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Title:
An integrated approach to bat convservation: applications of ecology, phylogeny and spatial modelling of bats on the Isle of Purbeck, Dorset

An integrated approach to bat conservation: applications of ecology, phylogeny and spatial modelling of bats on the Isle of Purbeck, Dorset

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A dissertation submitted to the University of Bristol in accordance with the requirements of the degree of PhD in the Faculty of Science

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Word count: 34,476

May 2008

Abstract

In response to the increasing loss of global biological resources there is an urgent need to identify and monitor all aspects of biodiversity. Tackling these challenges involves developing new approaches that integrate the many different fields of specialisation which occur in the field of biology. Combining techniques will lead to better informed management decisions in the future because more information on a species' ecological requirements will be provided. By studying bat communities on the Isle of Purbeck, Dorset, this study presents examples of how using different techniques at various times of the year can provide new information on the ecological requirements of different populations. Patterns of roost use and foraging behaviour of greater horseshoe bats (*Rhinolophus ferrumequinum*) at non-breeding roosts was established through continual monitoring, radio-tracking and diet analysis. These methods identified the importance of broad-leaved woodland within 4 km of the non-breeding roosts in spring and provided new information for this species at a time when it is not usually studied. Predictive habitat modelling and ground validation techniques were used to identify the potential distribution of Bechstein's bat (*Myotis bechsteinii*) maternity colonies. The approach showed the advantage of using knowledge-based models when there is little information available and the power of presence-only modelling when actual roost locations are known. The importance of different underground sites for swarming bat communities was assessed by surveying five sites within a small geographical area. High levels of inter- and intra-specific variation between underground sites were observed and the importance of conserving a range of different sites was identified. Finally, molecular techniques were used to identify the population structure, demographic history and genetic diversity of *R. ferrumequinum* across its Palaearctic range. Mitochondrial DNA was sequenced for 161 individuals at 45 different locations, including samples from the Isle of Purbeck, and compared to microsatellite data that were already available. Analyses identified high genetic diversity in east and west Asia with relatively low genetic diversity in Europe. Two colonisation events were identified in Europe, one before the Last Glacial Maximum from a west Asian refugium and one after it from southern European refugia. These results revealed the importance of sampling across a species' natural range and using different molecular markers in phylogenetic analysis. Overall, the research showed how an evidence-based, scientific approach can be used to develop conservation strategies for a range of protected bat species in an area of considerable biodiversity interest.

Acknowledgements

First, I would like to thank my supervisor, Gareth Jones, for all of his support and guidance over the course of my PhD. I would also like to thank David Bullock for introducing me to bats and for turning the 'Bats in the Landscape Project' into a PhD opportunity. I am also grateful to Stephen Rossiter for giving me the opportunity to carry out molecular work on the greater horseshoe bat samples. I owe special thanks to Angela Peters for all of the help she gave me during this project, including working tirelessly on SITA applications!

I am most grateful for the support I received from Phil Richardson, the National Trust wardens, Dorset Bat Group and all of the volunteers who helped with my fieldwork. In particular, I would like to give special thanks to Jan Freeborn, Sally Humphreys and Phil Smith for all of the spare evenings they gave up to help me. I would also like to thank all the landowners who give permission for me to work on their land, especially David and Ben Whitehouse.

At the University of Bristol, I would like to thank all past and present members of the Batlab but most notably Sarah Harris for her help both in the lab and in the field. Special thanks should go to Risha Patel and Steve Abolins for all of their support and advice. Finally, I am indebted to my mother for her unwavering (including financial) support. She may now finally see her son get a job after almost nine years of being a university student.

Bats were caught and sampled under Natural England licences. This project was financially support by the SITA Trust, The National Trust, Dorset Wildlife Trust, Natural England, Mammals Trust UK, BP and Community First.

Author's declaration

I declare that the work in this dissertation was carried out in accordance with the Regulations of the University of Bristol. The work is original, except where indicated by special reference in the text, and no part of the dissertation has been submitted for any other academic award. Any views expressed in the dissertation are those of the author and in no way represent those of the University of Bristol.

Jonathan Richard Flanders

Date: 20/10/06

Contents

List of tables, figures and equations

General Introduction

Protecting biodiversity

Biological diversity, or 'biodiversity' is the result of evolutionary events and processes over the last four billion years and can be defined as the variety and variability of life on Earth (Cox, 1993). It is complex and ever changing with interactions occurring across all levels of biological organisation including landscapes, communities, populations, species, individual organisms and genes (Noss, 1990).

At present global biodiversity is under threat because the Earth's biota is undergoing a period of unprecedented mass extinction as a direct result of human activities altering the environment (UNEP, 2007). In 1992 the United Nations Environment Programme responded to this crisis by proposing an international treaty to conserve and protect biodiversity. 'The Convention on Biological Diversity' was signed in Rio de Janeiro by 189 countries and was the first time in which international law has recognised that the conservation of biodiversity is an integral part of the Earth's (including mankind's) survival (UNEP, 1994). The treaty established clear objectives for countries to identify and monitor all aspects of biodiversity and to develop new, integrated approaches for the management of the earths biological resources (UNEP, 1994).

Conservation biology

First recognised as an emerging scientific field in the United States of America in the 1970s and 1980s, conservation biology is a multidisciplinary science that aims to conserve and protect biological diversity (Primack, 1993). Conservation biology plays an important role in protecting biodiversity by bridging the gap between politics, research and wildlife management so that the best habitat recommendations can be made and enforced. Conservation biology is commonly regarded as a crisis discipline, and conservation biologists often have to focus on rapidly changing ecosystems and provide management recommendations before all the details of a species' ecological requirements are fully known (Soule, 1985, Chapin et al., 2000). A fundamental concept of this process is in understanding how and why a species is found in a given area, how it has been maintained by natural processes in the past and what can be done to prevent

further population loss (Cox, 1993). Only by understanding all the risks faced by different populations and the mechanisms surrounding past extinctions will any progress be made in reducing the current loss of biodiversity. This poses considerable problems for conservation biologists because many species are going extinct before they are being discovered and the majority of areas have been altered to such an extent that they do not represent fully functioning ecosystems anymore (May, 1994, McCann, 2001). Conservation biologists therefore need to develop new ways in which to study different organisms so that as much information about their ecological requirements can be ascertained within a relatively short period of time.

New approaches to conservation biology

'Integrative biology' is a relatively new term used in science and has many different meanings (Ripoll et al., 1998, Wake, 2001). Overall it is used to describe the application of multidisciplinary approaches to research in order to provide a better understanding of different levels of biological complexity (Wake, 2003). How this is achieved greatly depends on what aspect of biology is being studied and the research goals of the investigator involved. Multidisciplinary approaches can involve cross-disciplinary co-operation (e.g. using expertise from the fields of physics, chemistry, biology, maths, engineering, etc.) or bringing together specialists within the same discipline (e.g. combining behavioural, ecological and genetic information) to answer a specific question.

Although the term 'integrative biology' has only been recently used in science it has been practiced for a very long time. The most famous example of an integrative biologist, or 'naturalist' as they were commonly referred to then (Pearse, 2003), was Charles Darwin. By using and integrating a range of different skills such as morphology, development, behaviour, physiology and ecology Darwin demonstrated his theory of evolution through natural selection (Wake, 2003). Over the last hundred years the role of the naturalist has diminished due to advances in technology creating greater specialism within the different fields of science. Clearly, new fields of expertise are necessary to address specific aspects of broader scientific questions but without integrating this knowledge with other areas of research it is impossible to understanding the full importance of the findings within their true biological context.

Combining different fields of interest in order to maximise the level of understanding has been used to great effect in a wide range of studies including research focussing on biodiversity (Wake, 1995). Examples of this include combining physiology, ecology and molecular biology to determine the development and mating success of dragonflies (*Libellula* spp.) (Marden et al., 1998); the use of bioacoustics, behaviour and molecular techniques to identify two cryptic species of pipistrelle bat (*Pipistrellus* spp.) (Jones and van Parijs, 1993, Barratt et al., 1997) and produce correct management recommendations for both species (Davidson-Watts and Jones, 2006, Davidson-Watts et al., 2006); the integration of phylogenetics, ecology and behaviour to help understand the biogeographical history and current distribution of Madagascar's *Triaenops* bats (Russell et al., 2007) and combining biogeography and ecology to estimate current levels of species richness (Wiens and Donoghue, 2004).

Bats and biodiversity

Bats are the second most species-rich mammalian order in the world and represent significant contributors to biodiversity (Altringham, 1996, Racey and Entwistle, 2003). Over the last 100 years bat populations have been in decline with many species ranges contracting or some even going extinct (Stebbings, 1988). This is of great concern because bats fill important roles in different ecosystems by acting as predators to a wide range of insects and other species as well as pollinating and dispersing the seeds of many species of plant (Altringham, 1996, Racey and Entwistle, 2003). They are also the only mammalian order naturally capable of flight and provide excellent evolutionary examples of adaptive radiation, convergent and divergent evolution (Jones and Teeling, 2006, Jones and Holderied, 2007), predator-prey interactions (see: Altringham, 1996 for review) and the use of ultrasound in echolocation (Griffin, 1958).

Threats to bats closely mirror those faced by other biota and can be directly associated with the increase of human demands on the ecosystem. Bats are extremely effective bio-indicators for conservation biologists measuring the impact of man on the environment as their relative abundance, species richness, ecological diversity, vulnerability to disturbance can be relatively easy to monitor over successive years (Fenton et al., 1992).

Habitat destruction through agricultural intensification, overharvesting, pesticide use and the loss of roosts through disturbance and exclusion have had major impacts on populations (Stebbings, 1988). Bats are especially vulnerable to extinction because many species exhibit natural rarity, slow population growth and a high dependence on specific habitat types (Racey and Entwistle, 2003). Habitat fragmentation is a great concern because the loss of habitat corridors between different colonies often leads to small, genetically isolated populations which can lead to detrimental effects such as inbreeding depression, lack of resistance against disease and inability to survive stochastic events (Bright, 1993).

Bat conservation has been successful in highlighting the threats faced by bats and protecting many species. The creation of the Chiroptera Specialist Group by the International Union for Conservation of Nature (IUCN) and their subsequent publications of action plans for Megachiroptera (Mickleburgh et al., 1992) and Microchiroptera (Hutson, 2001) have been important in outlining the conservation status of bats and providing guidance for management plans. There is still, however, considerable need for further research as there is a general lack of understanding surrounding the ecological requirements of many species of bat. More specifically, information is needed to identify:

1. The past distribution of species – if available this can be used to understand factors resulting in species' decline and may identify populations of historical importance/rarity that could have a greater conservation priority.
2. The current distribution of species – without knowing where a species is found there can be no way of detailing any aspects of its ecology.
3. Habitat requirements – by gathering information throughout a species' lifecycle a full appreciation of the threats faced by a species can be learnt.
4. Threats to a species/reasons for decline – through the identification of the actual and potential threats faced by a species more accurate management advice can be given.
5. Conservation management plans – these should encompass all of the above and provide effective advice to protect and enhance a species' range.

Purpose of research

It is essential that future research in conservation biology utilises advances in technology to keep developing new integrative approaches in order to face the deepening biodiversity crisis. This study aims to highlight how different techniques, including molecular analyses, computer modelling and field surveys can be used to achieve this. One further aim of this study is to show the need for future work to investigate all aspects of a species' ecological requirements because without a full understanding of all the processes influencing population viability, accurate management recommendations cannot be made.

The work presented in this thesis focuses on the ecology of different species of bat found in the UK. All bats in the UK are protected by law due to their rapid decline over the last 100 years and are part of various local and national conservation initiatives. Out of the 17 resident species of bat in the UK, three species are classified as Vulnerable by the International Union for Conservation of Nature (IUCN, 2007) (*Rhinolophus hipposideros, Myotis bechsteinii and Barbastella barbastellus*) and seven species are subject to UK Biodiversity Action Plans (UK BAP) (*Rhinolophus ferrumequinum, Rhinolophus hipposideros, Myotis bechsteinii, Barbastella barbastellus, Nyctalus noctula, Plecotus auritus and Pipistrellus pygmaeus*) (Anon, 2007). Two species protected by BAPs (*R. ferrumequinum* and *M. bechsteinii*) feature extensively in this thesis.

Fieldwork was carried out on the Isle of Purbeck, Dorset unless stated otherwise. The Isle of Purbeck covers approximately 155 km^2 of land and is characterised by its Jurassic coastline, two chalk down ridges, extensive heathland, ancient semi-natural woodlands and large areas of pastureland. The Isle of Purbeck is a significant area for biodiversity because it supports over 200 species of national or local conservation importance (Purbeck Biodiversity Steering Group, Anon, 1998). Approximately 22% of the district is protected under UK (Site of Special Scientific Interest) and European conservation designations (Special Protection Areas, Special Areas of Conservation). Nationally, it contains approximately 5% of both the national heathland and reedbed resource even though it only covers a small area of the UK land surface (0.3% of England and Wales) (Purbeck Biodiversity Steering Group, Anon, 1998). Although no

major bat surveys have been carried out on the Isle of Purbeck since the 1960's, historically it has had a rich bat fauna, with fifteen of the seventeen resident species of bat in the UK having once been recorded (Dorset Environmental Records Centre [DERC] records). Abandoned inland quarries found all along the south Purbeck ridge are known to be used as hibernacula for at least ten species of bat, including the endangered greater horseshoe bat (*Rhinolophus ferrumequinum*) and, until the 1980's, the greater mouse-eared bat (*Myotis myotis*).

Thesis outline

Each chapter in this thesis represents a different study focussing on one aspect of bat ecology where there was hitherto limited information available. The information gained from each study will go towards the five main conservation objectives in protecting and conserving different species of bat, and management advice is developed where applicable.

Chapter two investigates the ecology of the greater horseshoe bat (*Rhinolophus ferrumequinum*) using a non-breeding roost on the Isle of Purbeck. By studying patterns of roost use, foraging behaviour and diet this chapter aims to provide new information on the importance of non-breeding roosts at a time of year where little is known about the life history of this species.

Chapter three uses ecological niche models to predict the distribution of Bechstein's bat (*Myotis bechsteinii*) maternity colonies in the south of England. Currently little is known about this rare species of woodland bat and only a small number of maternity roosts have been found. By using computer modelling and ground validation methods this chapter aims to identify this species' distribution on the Isle of Purbeck and provide a framework for investigations searching for rare species in the future.

Chapter four investigates the importance of underground sites for autumnal swarming bat communities. Although the importance of swarming sites for bats is now being recognised, very few studies have gone beyond describing what bat communities are found using individual sites. This chapter aims to assess the importance of structurally

different quarries within a small geographical area for bats so that improved management advice can be produced to protect these sites in the future.

Chapter five aims to understand what impact the Last Glacial Maximum (LGM) had on the distribution and colonisation events of *R. ferrumequinum* across its Palaearctic range. By comparing microsatellite data already available (Rossiter et al., 2007) to the results obtained by sequencing mitochondrial DNA collected from 45 localities ranging from the UK (including the Isle of Purbeck) to Japan, this study aims to show the importance of using different molecular markers in understanding the influence of different regions in the colonisation process, especially within Europe. The results also place the genetic diversity of *R. ferrumequinum* on Purbeck in a wider geographic context.

Roost use, ranging behaviour and diet of the greater horseshoe bat *Rhinolophus ferrumequinum* in Dorset

Summary

In response to the rapid decline of the greater horseshoe bat (*Rhinolophus ferrumequinum*) in Europe conservation efforts have previously focussed on protecting maternity roosts and hibernacula. Currently, there is little information available on the ecology of this species outside of these two main periods. This study investigates patterns of roost use, ranging behaviour, habitat use and diet of *R. ferrumequinum* using data collected from two non-breeding roosts over three years on the Isle of Purbeck, Dorset. The results show that the non-breeding roosts were predominantly used during the spring and autumn and acted as transitional roosts between maternity roosts and hibernacula. Compositional analysis of ranging behaviour collected in the spring revealed that *R. ferrumequinum* actively selected to feed in grazed pastures and broad-leaved woodland compared to any other available habitat. Diet analysis revealed that there was little difference between the diet of individuals using one of the non-breeding roosts and the nearest known *R. ferrumequinum* maternity colony. Conservation recommendations for *R. ferrumequinum* are discussed and highlight the need to protect/enhance the levels of broad-leaved woodlands within 4 km of non-breeding roosts used in the spring.

Introduction

The greater horseshoe bat (*Rhinolophus ferrumequinum*) is one of only two species in the genus *Rhinolophus* found in the UK. Both are of conservation concern and listed in the IUCN Red List of Threatened Animals (IUCN, 2007).

Although considered as 'not threatened' across its natural range (Hutson, 1993, Anon, 1995), *R. ferrumequinum* populations have declined significantly throughout northern Europe over the last hundred years (Hutson, 2001). *R. ferrumequinum* is considered to be 'endangered' in Europe (Hutson, 1993) and is listed under Appendix II of the Bonn Convention (and its Agreement on the Conservation of Bats in Europe, 1994), Appendix II of the Bern Convention (and its appropriate Recommendations) and Annexes II and IV of the EC Habitats and Species Directive. In the UK, where *R. ferrumequinum* is found on the northern edge of its geographical range (Csorba et al., 2003), it is protected under Schedule 2 of the Conservation (Natural Habitats, etc.) Regulations (1994), Schedule 5 of the Wildlife and Countryside Act (1981) (as amended) and is a UK Biodiversity Action Plan (UK BAP) priority species.

Originally a cave dwelling species, utilising underground sites for both breeding and hibernating (Ransome, 1990), *R. ferrumequinum* is mostly found using old, large buildings such as barns, stables and lofts during the summer when females exhibit strong philopatric behaviour and form maternity colonies (Ransome, 1980). As females tend to return to the maternity colony in which they were born *R. ferrumequinum* is especially vulnerable to disturbance to the site or surrounding area. Reasons for the decline of *R. ferrumequinum* are generally recognised to be through a combination of factors that include; habitat fragmentation (including farming intensification with the loss of small woodland and hedgerows), increased rates of disturbance including loss of caves, mines and buildings, and through the increased use of pesticides killing both bat and their prey (Stebbings and Arnold, 1987, Ransome, 1991, Duvergé and Jones, 1994, Harris et al., 1995, Longley, 2003).

Status in the UK

Although it is difficult to estimate what the UK population of *R. ferrumequinum* was over 100 years ago (Stebbings and Arnold, 1987, Stebbings, 1988, Ransome, 1989, Stebbings and Arnold, 1989, Ransome, 1990, Yalden, 1999), there is a general agreement that their numbers have shown a significant decline. Currently, there are only 35 recognised maternity/all-year roosts and 369 hibernation sites in the UK (Longley, 2003) with populations being confined to south-west England and south Wales (Harris et al., 1995). Population estimates of *R. ferrumequinum* in the UK range between 4,000 and 6,600 individuals (Anon, 1995).

In response to the rapid decline of *R. ferrumequinum* in Europe a large number of studies have been carried out focussing on many aspects of the ecology and behaviour of this species. Work initially focussed on population changes (Stebbings and Arnold, 1987, Ransome, 1989), roosting requirements during the summer (Ransome, 1973, 1978, 1998) and winter (Ransome, 1968, 1971, 1990, Park et al., 1999, 2000a, Ransome, 2002) and diet (Jones, 1992, Duvergé and Jones, 1994, Ransome, 2000). Since radio-transmitters have been made small enough to allow radio-tracking to take place, a number of studies (mainly at maternity colonies) have looked at the ranging and foraging patterns of *R. ferrumequinum* (Stebbings, 1982, Jones and Morton, 1992, Duvergé and Jones, 1994, Jones et al., 1995, Duvergé, 1996, Billington, 1999, 2000a, 2000b, Robinson et al., 2000, 2002, Bontadina, 2002, Billington, 2003, Duvergé and Jones, 2003). More recently, studies have been carried out looking into genetic variation and population structure (Rossiter et al., 2000, 2007) including the effects of inbreeding (Rossiter et al., 2001) and mate choice (Rossiter et al., 2005).

At present, the majority of research and conservation efforts targeting *R. ferrumequinum* have focussed on maternity roosts and their surrounding areas. This is not surprising considering the importance of maternity roosts in determining a population's reproductive success. English Nature's Species Recovery Programme proved successful in influencing the agricultural practices and landscape management around key maternity roosts in Devon, Somerset and Cornwall (Longley, 2003). However, conservation work to protect non-breeding roosts has largely been neglected. This is despite the *R. ferrumequinum* UK BAP stating that future research and monitoring

should "promote research to assess the importance of sites used by small numbers of bats and develop and implement a strategy for their conservation" and to "investigate the rate of loss of minor sites and their importance to the population structure" (Anon, 1995).

The identification and protection of non-breeding roosts is important because they have the potential to act as 'transitional roosts' for individuals commuting between maternity roosts and hibernacula. Non-breeding roosts may also link different colonies and facilitate gene flow between them. The decline and loss of *R. ferrumequinum* colonies has meant that small, remnant populations have remained across northern Europe leaving many isolated from one another (Ransome and Hutson, 2000, Bontadina, 2002). Previous work has shown there is evidence of inbreeding depression (Rossiter et al., 2001) and limited migration events (Rossiter et al., 2006, 2007) among colonies in the UK, highlighting the importance of facilitating gene flow to maintain population health.

The major aim of this study was to investigate habitat use of *R. ferrumequinum* around a non-breeding roost in Dorset. By focussing on the habitat preferences of bats in the spring and autumn, and from a type of roost that is not usually studied, a better understanding of the ecology of *R. ferrumequinum* can be gained. New aspects of this species' behaviour will allow for improvements to be made to current conservation strategies that will hopefully contribute to the BAP target of increasing the current population of *R. ferrumequinum* by 25% by 2010.

The objectives of this study were:

1. To identify the principal habitats used by foraging *R. ferrumequinum* around their non-breeding roost site.
2. Determine the range at which they forage.
3. Compare the diet of *R. ferrumequinum* at the non-breeding roost to that of bats using a maternity roost.
4. To compare these findings with previous studies carried out at maternity roost sites and identify any differences that may influence management recommendations.

Methods

Study area

This study was carried out over three years (2005 – 2007). The non-breeding roost chosen for the project was an ancient watermill called Boar Mill situated on the Isle of Purbeck, Dorset (50°38.22' N, 2°03.25' W) (Fig. 1). Boar Mill was first identified as a roost in 2000 when one *R. ferrumequinum* was found using the site. Between 2000 – 2003 a maximum of two bats were recorded at Boar Mill at any one time. In 2003 the upstairs windows were boarded up in order to encourage bats to use the site. Surveys carried out as part of this study have identified that the number of *R. ferrumequinum* using this site has increased with a maximum of 16 bats counted at any one time.

Boar Mill is situated at the bottom (maximum elevation 30 m) of a natural break in the north Purbeck ridge (between 100 – 190 m high), one of two natural features that divide Purbeck (Fig. 2). The surrounding area is dominated by agriculture with the majority of fields used for pasture or arable crops. Known hibernacula are situated within 5 km south of the Mill (Fig. 1). The closest known maternity roost to Boar Mill, and the site chosen for dietary comparison, is situated in a disused building owned by the Vincent Wildlife Trust at Bryanston School, Blandford Forum (50°51.45' N, 2°10.51' W). Bryanston is approximately 30 km north of Boar Mill (Fig. 1).

Historically, the Isle of Purbeck had one of the UK's largest *R. ferrumequinum* maternity colonies (Stebbings and Arnold, 1987). The bats used a series of outbuildings at a Victorian estate house in Creech until May 1953 when the outbuildings were sprayed with a 1% solution of the pesticide *gamma*-Hexachlorocyclohexane (HCH) (commonly known as lindane) (Stebbings and Arnold, 1987). Lindane has now been banned in many countries as the highly toxic properties that go far beyond its original purpose have now been recognised. A large number of bats were apparently killed in the first year of spraying with subsequent individuals dying as they returned to the roost during the years that followed (Stebbings and Arnold, 1987). This incident effectively eradicated this maternity colony of bats.

Figure 1. Map showing the location of the *R. ferrumequinum* non-breeding roost at Boar Mill, the abandoned maternity colony at Creech and the maternity colony at Bryanston. Known areas containing *R. ferrumequinum* hibernacula are also shown. The Isle of Purbeck is highlighted in grey.

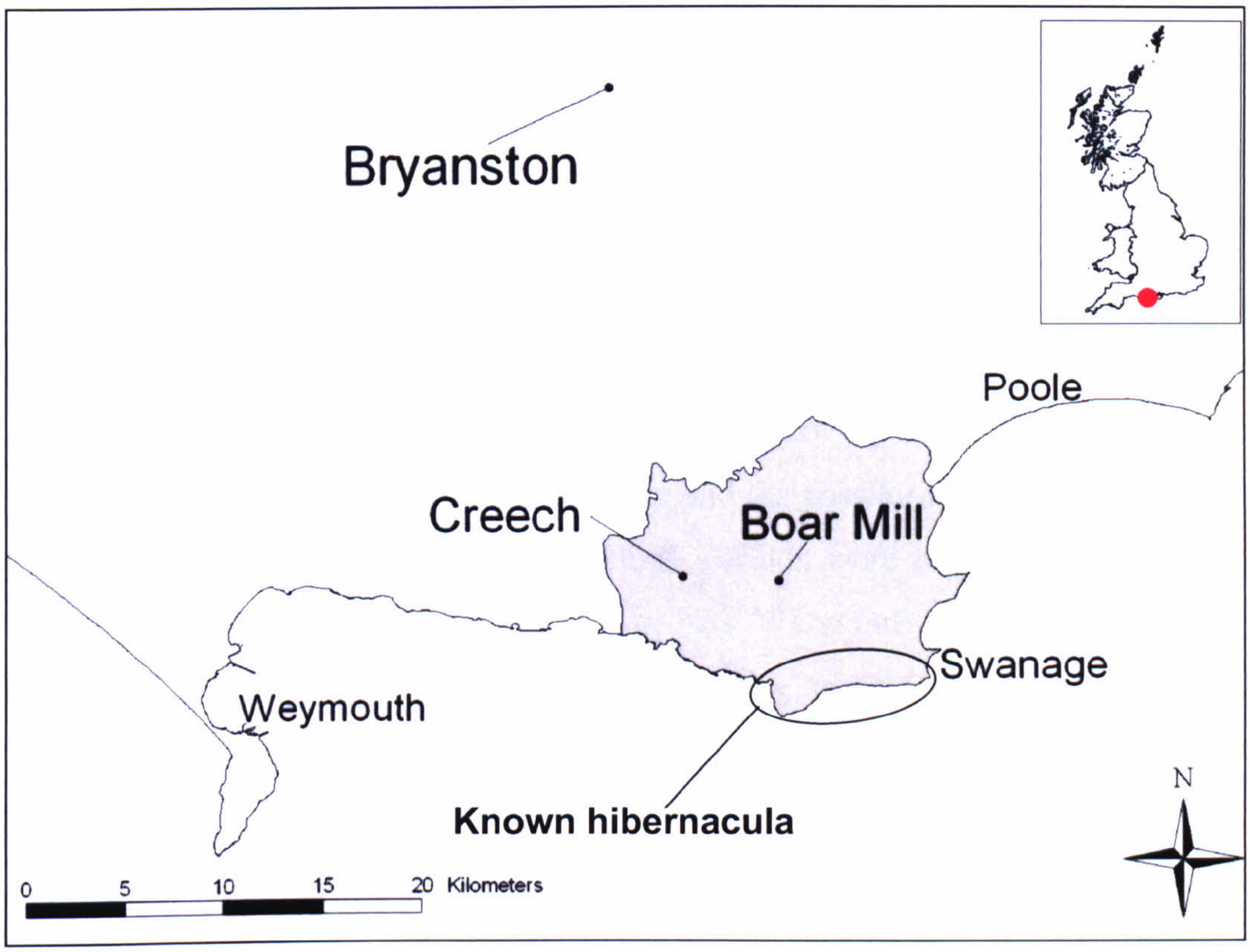
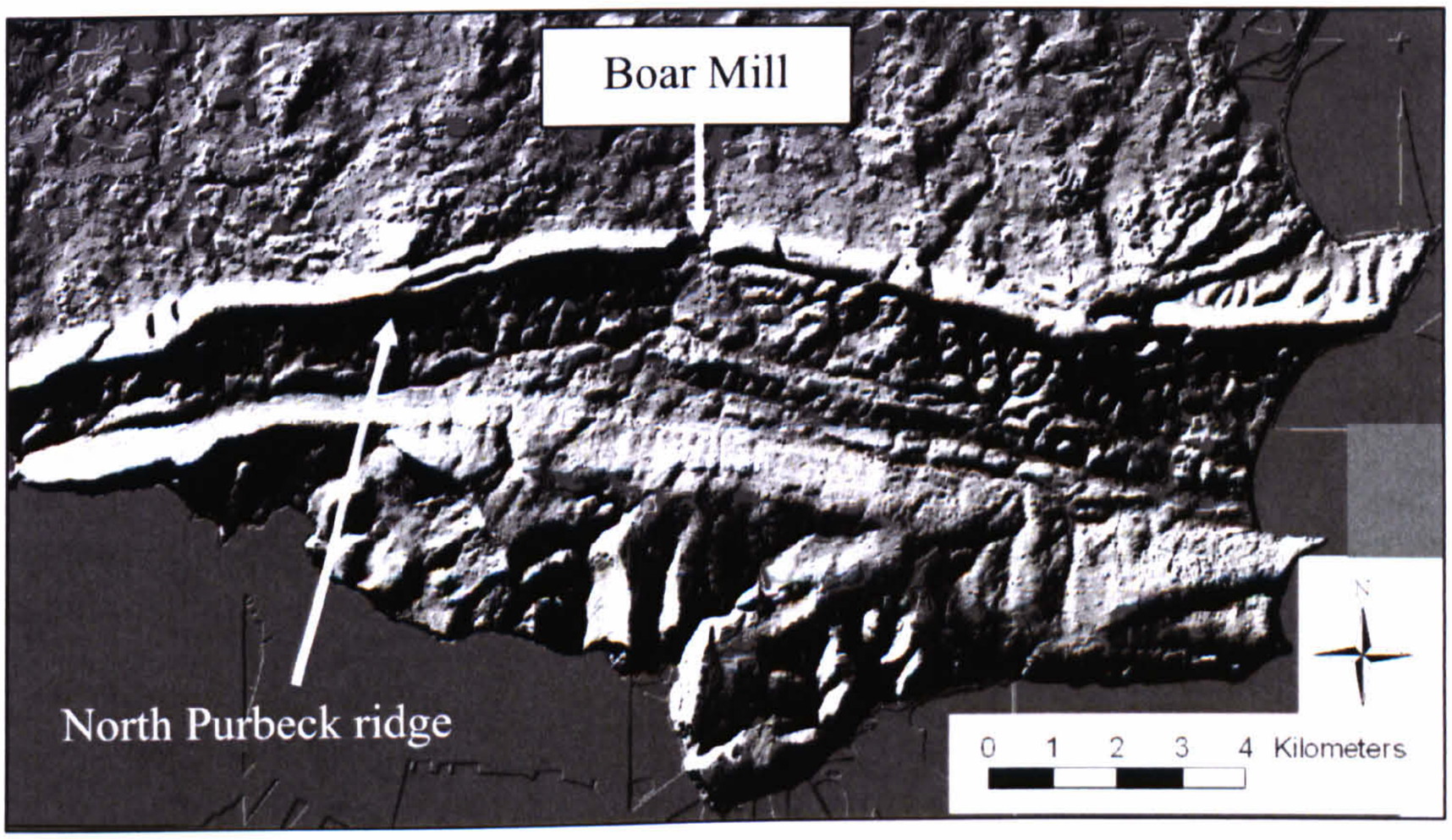


Figure 2. Map showing the topography of the Isle of Purbeck including the position of Boar Mill in context to the natural break in the north Purbeck ridge.



Roost use, foraging strategies and ranging behaviour

Emergence counts of *R. ferrumequinum* were made at Boar Mill every two weeks in order to identify patterns of roost use throughout the year. No catches were made except to apply radio-transmitters in order to minimise the disturbance to the bats at their roost. Once all of the bats had exited Boar Mill, identified by a twenty minute period of no activity after the last bat was seen to leave, the roost was entered in order to check no bats were left in torpor and to collect faeces for dietary analysis.

Bats were caught and handled during the day at Boar Mill using handnets under English Nature licence. Bats were only caught when there were eight or more individuals present in order to allow for around four individuals to be fitted with radio-transmitters, after allowing for the probabilities of recapture and the possibility that some individuals my be too light for tagging. No two catching periods were held within a two week period in order to reduce the disturbance to the bats. When individuals were caught their biometric details were recorded (sex, age, breeding condition, forearm length and weight) and they were fitted with 4.8 mm aluminium rings issued by the Mammal Society. Ringing the bats was necessary to identify individuals over successive years so that no bat was radio-tracked twice. Radio-transmitters weighing between 0.7g and 1.2g (manufactured by Biotrack Ltd, Wareham, Dorset, UK) were used. These were attached to the area between the scapulae using Skin Bond (Pfizer Inc.), a form of biodegradable glue, after clipping the bat's fur. Radio-transmitters were chosen so that they were within 5% (mean 4.8%) of the animals body mass (Kenward, 1992). All radio-transmitters had an average life expectancy of two weeks and usually became unattached from the bat before they stopped transmitting a signal. The bats were radio-tracked using a SRX 400 receiver (Lotek Engineering Inc., Newmarket, Ontario, Canada) between March and April 2005 and with an Australis 26K scanning receiver (Titley Electronics Ltd., Ballina, Australia) thereafter. Both receivers were used with the same three-element Yagi collapsible antenna (Mariner Radar, Lowestoft, UK).

Due to the man-power and equipment that was available, the triangulation method (Kenward, 1992) was not used. As an alternative, following the methods outlined by Jones et al. (1992) and Duvergé (1996), individual bats were followed from dusk to dawn to maximise data collection. By following an individual bat, peak signals (with

corresponding bearings noted using a compass) were taken at regular intervals (between 5 – 10 minutes) depending on how fast the bat was moving to allow for an approximation of the bats' flight path to be identified (Robinson et al., 2000). The tracker's location was recorded to a ten figure grid reference (British National Grid) using a GPS (Garmin e-trex, 5 – 15 m accuracy) and the confidence of the bat's location was assigned an accuracy of 1 – 3 (50, 100 & 150 m) according to the strength of the signal and the gain used on the receiver. The accuracy of these classes was determined *a priori* by carrying out a field test involving tracking a volunteer in different habitats within the study site. Calibration was necessary as the variability of the signal strength depended on a number of factors that include the density of vegetation, topography and distance between transmitter and receiver.

Although individuals were tracked on the first night of having their transmitters fitted, in order to gain an overall impression of where they were foraging, these results were not used in any analysis. This was done in order to let the bat acclimatise to the transmitter in the event that the initial disturbance altered the bat's foraging behaviour. When a bat was being tracked there were occasions when its signal would be lost. When this occurred the bat would be considered 'lost' until its signal could be located. If a bat remained lost for a period of more than 45 minutes, that night's data would not be used in the final analysis because the complete pattern of movements throughout the night could not be identified.

Analysis of ranging behaviour and habitat preference

Using observer location, bearing and signal strength, the bat's location was digitised using the GIS software ArcView 3.2 (Esri Inc.) and Distance/Azimuth Tools (version 1.6) extension (Jenness Enterprises) and then exported into ArcGIS 9.2 (Esri Inc.). Once digitised, all radio-tracking data were analysed in Ranges 7 (Anatrack Ltd., Wareham, Dorset, UK).

To calculate foraging areas a combination of minimum convex polygons (MCP) (Kenward, 1992) and cluster analysis (Kenward, 1987) was used. Since the introduction of more robust analyses such as Kernel and clustering analysis, MCPs have been made largely redundant (Kenward, 2001). This is because MCPs have a high likelihood of

producing biases when estimating the principal habitat types used by foraging bats as they cannot distinguish between foraging areas and commuting routes (Kenward, 1987, Harris et al., 1990, Burgman and Fox, 2003). MCPs have, however, been the most widely used method in the past (e.g. Duvergé, 1996, Billington, 1999, 2000a,b, 2002, 2003) so were included in this study so that the results can be used for comparative analysis. Other analysis, such as harmonic mean and Kernel analysis, could not be used as these techniques use parametric assumptions when interpreting the location of bat fixes. Since the bats' locations could not be recorded at regular time intervals due to the constraints of using the homing-in method it was not possible to provide the independent location density estimators needed for such tests (Kenward, 2001). By continually radio-tracking a bat in the field, less fixes of the bats location are made when it is moving quickly over large areas compared to when it is moving short distances over a long period of time. Cluster analysis was considered to be the best alternative for distinguishing foraging areas from commuting routes because the bats were observed using small pockets of habitat for extended periods of time (Davidson-Watts et al., 2006). To determine the percent cluster cores needed to exclude commuting routes from foraging areas the utilisation distribution discontinuities were analysed from 100% in decreasing 5% intervals to see at which point the core foraging range stop decreasing exponentially (Davidson-Watts et al., 2006).

To calculate habitat preference, the foraging area of each bat was compared to the habitat available using compositional analysis. This was carried out using Compositional Analysis Plus Microsoft Excel tool 6.2 (Smith Ecology Ltd., Abergavenny, UK) according to the methods of Aebischer et al. (1993). Unlike the examples set by Aebischer et al. (1993), individual bat's MCPs were not used to calculate available habitat because the majority of the bats were foraging in a small area with little habitat variability. If compositional analysis was carried out according to Aebischer et al. (1993) (e.g. Davidson-Watts et al., 2006) it would show that bats were choosing the habitats at random. This is because the analysis would effectively be comparing used vs. used habitats because there is not enough habitat variability between the MCPs and 90% cluster cores at $P = 0.05$ to show any habitat preference. For meaningful results, 'available habitat' should include areas that could be used, but are being avoided. Using individual MCPs and cluster cores meant that this would not have occurred in this study. Habitat availability was instead determined by creating one

single MCP by pooling all of the bats' positions (Russo et al., 2002). This effectively outlines the maximum foraging range of the bats and was seen as a more suitable alternative than using maximum range circles around the two roosts (Waters et al., 1999) due to the effect of the north Purbeck ridge in limiting the bats' movements in certain directions (personal observation). During the course of the study no individual was radio-tracked flying over the north Purbeck ridge with the natural gap at Boar Mill acting as the only link between the two sides. The north Purbeck ridge effectively makes the majority of habitat on the south side of the ridge 'unavailable' to foraging bats.

A land-use map was created in ArcGIS 9.2 to be used in the compositional analysis. Habitats were split up into eight general habitat types broadly taken from the Phase 1 habitat classification (JNCC, 2003) after being identified through field surveys (Table 1).

Table 1. Description of the eight habitat types used to classify the available habitat for compositional analysis.

Habitat type	Description
Broad-leaved woodland	Dominated by semi-natural broad-leaved woodland, plantation broad-leaved woodland, coppice woodland and orchards
Coniferous woodland	Coniferous plantations or coniferous woodland with less than 10% deciduous trees
Mixed woodland	Woodland with over 10% deciduous and coniferous trees
Pasture	Includes all areas that are grazed by farm livestock (e.g. cows and sheep) and recreational animals (e.g. horses)
Arable	Includes all areas used as part of arable crop rotation (including set-aside)
Scrub	Sites characterised by small shrubs and bracken
Grassland	Grassland not used for any agricultural practice
Heathland	Vegetation dominated by ericoids (heathers and bilberry), dwarf gorse species or lichens and bryophytes generally occurring on well drained acidic soils
Water	Includes both man-made and natural water features
Urban	Includes all villages and farmsteads

Diet

To understand the factors influencing a bat's foraging behaviour, a full appreciation of its prey choice is needed. Seasonal changes in diet can be detected and may help explain any changes in habitat use throughout the year (Shiel et al., 1997) and can allow for an assessment of whether there is evidence of prey selection (Swift and Racey, 1983).

Dietary comparisons were made between *R. ferrumequinum* using Boar Mill and the maternity roost at Bryanston. Bryanston was chosen for comparison as it is the closest known maternity roost to Boar Mill, has similar surrounding habitat, and will experience similar climatic variations that could influence the insect activity at the two sites during the course of the study.

Dietary analysis of insectivorous bats may be carried out through direct observation (Vaughan, 1976, Acharya and Fenton, 1992, Dunning et al., 1992), examination of faecal samples (Eckrich and Neuweiler, 1988, Lacki et al., 1995, Shiel et al., 1997), discarded prey remains (Laval and Laval, 1980, Davison and Zubaid, 1992) and the gut (Kunz, 1974, Whitaker and Black, 1976, Kunz et al., 1995) or through a combination of these methods (Griffith and Gates, 1985, Mcaney and Fairley, 1989, Jones, 1990, Fenton et al., 1993, Sample and Whitmore, 1993, Brooke, 1994, Duvergé and Jones, 1994, Arlettaz, 1996). Since *R. ferrumequinum* are endangered in the UK and stomach content analysis is lethal for the bats this method was not chosen. Initially, examining both faecal remains and discarded prey items was chosen to obtain dietary information for *R. ferrumequinum*. This was later revised to only looking at faecal remains as prey items could not be collected from Bryanston and only a small number of feeding perches were ever found around Boar Mill.

Collection of faecal samples

Faecal samples were obtained by positioning collection trays beneath the areas most frequently visited by bats. At Boar Mill collection trays were emptied twice per month with the remains sealed in an airtight bag and frozen until analysis. At Bryanston faecal samples were collected once a month by Mr. Colin Morris (VWT) and stored using the same method.

Analysis of faecal samples

Twenty faecal samples were picked at random and dissected from each collection period from each study site using the methods described in Shiel et al. (1997). Previous dietary analysis of *R. ferrumequinum* by Jones (1990) and Duvergé (1996) found that 16 faecal samples is a sufficient number to give a representative sample of the diet over this time interval. Twenty samples were chosen in this study due to the slightly longer collection periods at Bryanston. To check that 20 faecal samples was a satisfactory number of samples to analyse the cumulative number of taxa were plotted against the number of samples analysed.

Each sample was allowed to soak in hot water for at least one hour before being transferred to a petri dish. Two drops of glycerol were added to the sample which was then teased apart using fine dissecting needles. A drop of 70% ethanol was added to allow for the insect remains to be evenly smeared over a small section of the petri dish to form a thin layer of exoskeleton fragments. Insect remains were examined under a binocular microscope (GX XTL – 2I, 7 – 42x magnification; GM Optical, Suffolk, UK) and compared to the fragments of previously dissected and positively identified insects. To aid visual estimates the bottom of the petri dish was marked with a 1 x 1cm grid (Whitaker, 1988). Diet was quantified as the percentage volume of each prey in the diet because this is the most accurate technique of dietary analysis for this species (Duvergé, 1996). The % volume of each prey item was estimated to the nearest 5% (Jones, 1990) although occasional items that only constituted to 1 – 2% of any sample were recorded as such. The total % volume of each prey item for each collection period was calculated by taking the average value over all 20 samples analysed.

Data analysis

To compare for differences in species composition between the two sites multiple Wilcoxon signs ranks tests were carried out on each prey type using the mean % prey volume per month over the three year study period. To overcome the problems of multiple testing and its effect on Type II error (Dytham, 2003), a Bonferroni correction was applied and the original p-value adjusted for the number of comparisons ($\alpha = 0.05 / 9 = 0.006$).

Food niche breadth was measured using Simpson's index of diversity (1 – D) (Equation 1). Simpson's index of diversity was chosen over other measures such as Shannon's diversity index because it is less affected by species abundance and takes into account species richness. Simpson's index of diversity ranges between 0 and 1, with the greater the value indicating the greater the diversity. Two-way ANOVA (without replication) was used to assess variation between the two sites by testing the mean monthly diversity index after pooling the data for all three years. Food niche overlap was measured using Pianka's index (O_{jk}) (Pianka, 1973) (Equation 2). This index ranges between 0 and 1, with the greater value indicating the greater overlap. An index greater or equal to 0.60 is considered to show significant overlap (Zaret and Rand, 1971, Wallace, 1981).

Equation 1. Simpson's index of diversity = (1 – D)

Where:

$$D = \sum_{i=1}^{S} \frac{n_i(n_i - 1)}{N(N-1)}$$

n = the total number of organisms of a particular species;
N = the total number of organisms of all species;

Equation 2. Pianka's index (O_{jk})

$$O_{jk} = \frac{\sum_{i}^{n} p_{ij} p_{ik}}{\sqrt{\sum_{i}^{n} p_{ij}^2 \sum_{i}^{n} p_{ik}^2}}$$

O_{jk} = measure of niche overlap between species j and k;
p_{ij} (or p_{ik}) is the proportion of the food category i recorded in the diet of species j (or k);
n = the total number of food categories.

Results

Roost use

Over the three year study period a maximum of 16 bats were counted using Boar Mill at any one time. Activity patterns were not uniformly distributed with major peaks in activity seen in May 2005 and 2006 and in April 2007 with low use during the summer (Fig. 3). Smaller peaks in activity occurred in September over all three years.

Radio-tracking data

A total of 21 *R. ferrumequinum* were fitted with radio-transmitters between 2005 – 2007 (Table 2). Due to a combination of transmitter failure, difficulty in tracking individuals or bats moving away from the area (including seven individuals moving to Bryanston), nine individuals were excluded from the study as less than three full nights data were available per bat. It was not possible to obtain enough nights of data for any of the bats radio-tracked in September 2005 because they did not consistently use any specific foraging areas or roosts. From the limited data available at that time, the bats spent the majority of their time flying between different underground sites (presumably male mating territories), which may partly explain why their signals kept being lost.

Radio-tracking data from the 12 individuals with ≥ 3 full nights data (mean 3.9 ± 1.2 days) spanning 47 nights were used to calculate home ranges, foraging areas and habitat preference. The mean number of fixes per bat (± SD) was 184 ± 92.

Home ranges

Although all of the bats were caught at Boar Mill, radio-tracking revealed that while some individuals stayed faithful to this roost, others moved to another roost at Creech, approximately 5 km west of Boar Mill. While some bats used both roosts during the time they were being tracked, others used Creech exclusively. As it is not possible to determine whether these bats were using the same foraging areas before they were disturbed, the mean maximum nightly flying range (from roost to furthest point

travelled) was calculated depending on which roosts the bats used during the time they were being tracked (Table 3).

The mean maximum nightly foraging range from Boar Mill and Creech was 2.17 km and 2.44 km respectively (Table 3). This was not significantly different between bats using either roost (ANOVA, $F_{1, 13} = 0.28$, $P > 0.5$).

Foraging areas

Analysis of the utilisation distribution discontinuities from 100% to 20% in 5% intervals found that the core foraging ranges stop decreasing exponentially at 90%. It was decided that 90% cluster cores should be used in the analysis.

There was a high level of overlap in foraging areas using MCPs within and between years (Fig. 4). Mean foraging area for each bat, as calculated using their MCPs, was highly variable (Table 3. Mean = 148.10 ha, 95% CI 88.16 – 208.00 ha). Analysing foraging intensity within an individual's foraging range using 90% cluster cores revealed that the utilisation density was unevenly distributed with small areas being selected (Table 3. Mean = 49.42 ha, 95% CI 37.91 – 60.93 ha) (Fig. 5). Comparing the areas of the smaller 90% cluster cores to that of the larger MCPs found that the areas were significantly different (ANOVA, log-transformed data, $F = 18.22$, d.f. = 23, $P < 0.001$).

Combining the radio-tracking points for all the *R. ferrumequinum* tracked (n = 12) to produce a single MCP revealed that the overall habitat composition within 632 ha of land was: 28.7% pasture, 26.6% broad-leaved woodland, 16.1% arable, 8.2% mixed woodland, 6.7% urban, 5.8% scrub, 4.3% grassland, 1.6% heathland, 1.4% water and 0.7% coniferous woodland.

Habitat preference

Habitat composition of individual 90% core foraging areas ('used') was significantly different from the combined MCP ('available') and habitats were not being selected at random (weighted mean Wilk's $\Lambda = 0.01$, $\chi^2 = 51.00$, d.f. = 9, $p < 0.0001$, randomisation

p = 0.03). A ranking matrix of the most to least preferred habitat types (Table 4) was ordered as follows: pasture > broad-leaved woodland >>> mixed woodland > water > scrub > grassland > urban >>> coniferous woodland > heathland > arable (where >>> shows significant selection of preceding habitat to the habitat that follows it and > represents non-significant selection of one habitat compared to another). Compositional analysis reveals that *R. ferrumequinum* selected to forage in broad-leaved woodland and pasture over any other habitat during the course of this study. Coniferous woodland, heathland and arable were the least selected foraging habitats, although coniferous woodland and heathland were limited in their availability. In contrast, arable land was the third most dominant habitat type (16.1%) but was the least selected habitat (Fig. 6).

Figure 3. Mean monthly *R. ferrumequinum* emergence counts (± max & min count) from Boar Mill collected between 2005 – 2007.

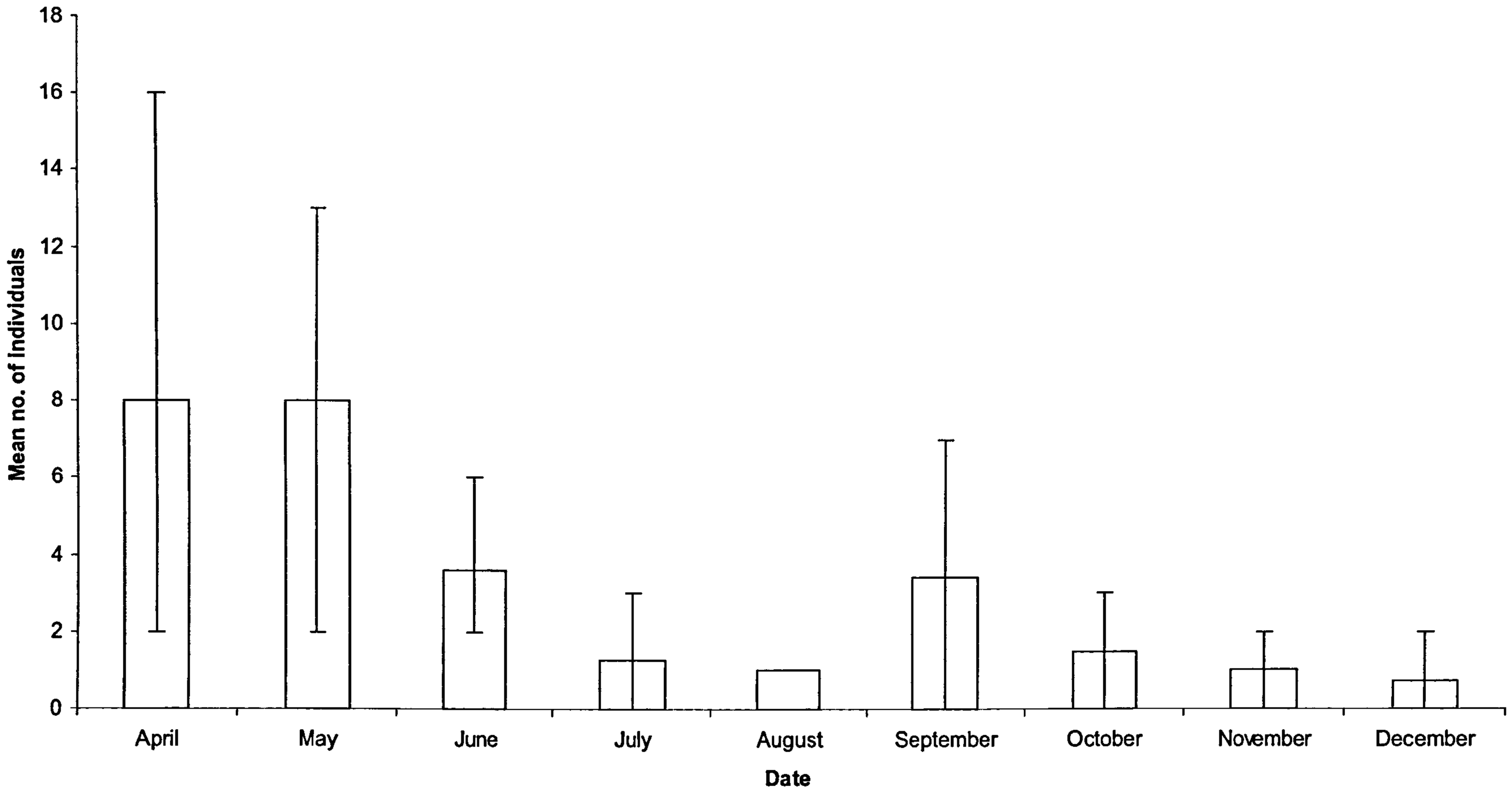


Table 2. Date of capture, sex, age, biometry (FAL = forearm length, BM = body mass), percentage ratio tag / body mass and number of full nights tracked of 21 *R. ferrumequinum* caught and radio-tracked at Boar Mill between 2005 – 2007.

Bat number	Capture date	Age	Sex	FAL (mm)	BM (g)	% tag weight / BM	No. full nights tracked
X1501	08/04/2005	Adult	F	53.6	19.50	5.1	2
X1502	08/04/2005	Adult	F	58.0	19.50	5.1	6
X1504	08/04/2005	Adult	F	56.8	18.50	3.8	4
X1519	12/05/2005	Adult	F	58.8	21.00	5.7	6
X1520	12/05/2005	Adult	F	54.8	19.50	5.1	1
E4807	12/05/2005	Adult	F	56.2	21.00	4.8	1
X1528	13/09/2005	Adult	F	57.0	23.00	5.2	0
X1530	13/09/2005	Adult	F	57.0	22.50	4.4	0
X1522	13/09/2005	Adult	M	54.6	18.50	3.8	1
X1531	13/09/2005	Adult	F	56.2	20.50	4.9	0
E9708	01/05/2006	Adult	F	56.8	18.00	5.6	3
X1532	01/05/2006	Adult	F	56.6	19.50	5.1	4
X1536	01/05/2006	Sub-adult	F	56.5	18.50	3.8	3
X1537	01/05/2006	Adult	F	55.9	21.50	4.7	3
X1538	01/05/2006	Adult	F	55.6	20.50	4.9	3
X1568	12/04/2007	Adult	F	56.9	21.00	5.7	0
X1569	12/04/2007	Adult	F	57.0	19.50	5.1	0
X1529	12/04/2007	Adult	F	56.2	21.50	5.6	5
X1571	12/04/2007	Adult	F	55.9	19.00	5.3	3
X1523	12/04/2007	Adult	F	55.8	18.00	3.9	3
X1521	12/04/2007	Adult	F	55.9	18.50	3.8	4

Table 3. Individual and mean (± 95 % CI) size of foraging areas using 100% minimum convex polygons (MCPs) and 90% cluster cores, and maximum distance flown from roost to foraging area*.

Bat number	Foraging area (ha) using		Maximum distance from roost to foraging area (km)	
	100% MCP	90% Cluster	Creech	Boar
X1502	40.22	14.24	-	0.95
X1504	84.25	40.07	-	1.41
X1519	356.29	72.14	-	2.21
X1521	149.66	39.25	2.55	-
X1523	47.66	37.35	0.61	-
X1529	87.77	53.62	1.54	-
X1532	103.91	64.00	1.85	-
X1536	206.88	44.37	3.49	2.93
X1537	127.95	83.45	1.96	-
X1538	229.69	56.25	3.5	2.8
X1571	95.51	43.04	2.73	-
E9708	247.13	45.22	3.76	2.73
Mean	148.10	49.42	2.44	2.17
95% CI	88.16 – 208.00	37.91 – 60.93	0.51 – 2.00	1.31 – 3.03

* Maximum distance travelled from roost to foraging area was only calculated using the roost(s) used by each bat during the period in which they were being radio-tracked.

Figure 4. Minimum convex polygons of 12 *R. ferrumequinum* radio-tracked on the Isle of Purbeck in 2005 (blue), 2006 (red) and 2007 (green). The two roost sites used during the tracking period, Boar Mill and Creech, are shown by red and white stars respectively.

Figure 5. Minimum convex polygons and 90% cluster cores of *R. ferrumequinum* in (a) 2005 (n = 4), (b) 2006 (n = 5) and (c) 2007 (n = 3). 90% cluster cores are shown within MCPs using the same coloured outline. The two roost sites used during the tracking period, Boar Mill and Creech, are shown by red and white stars respectively.

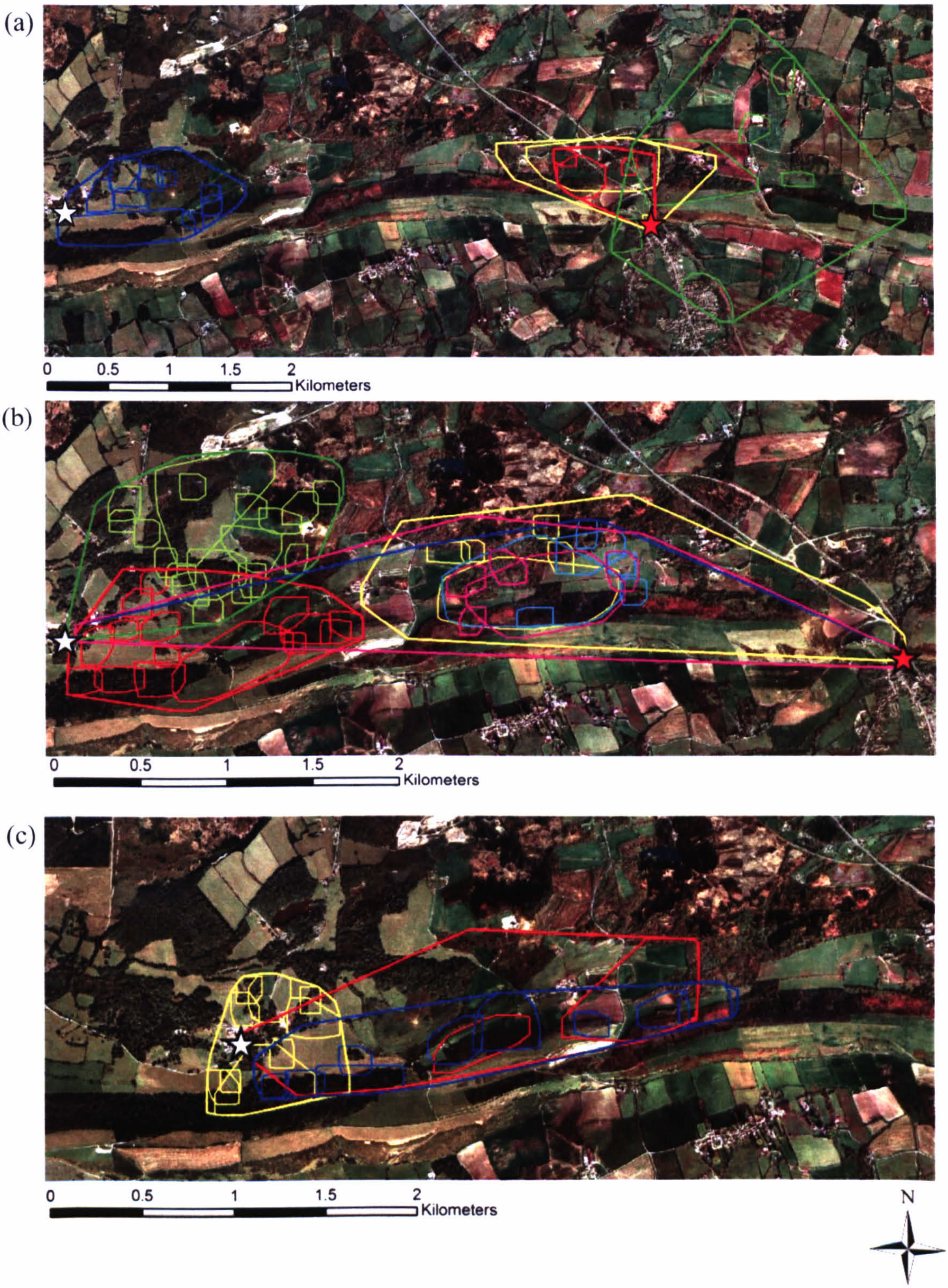
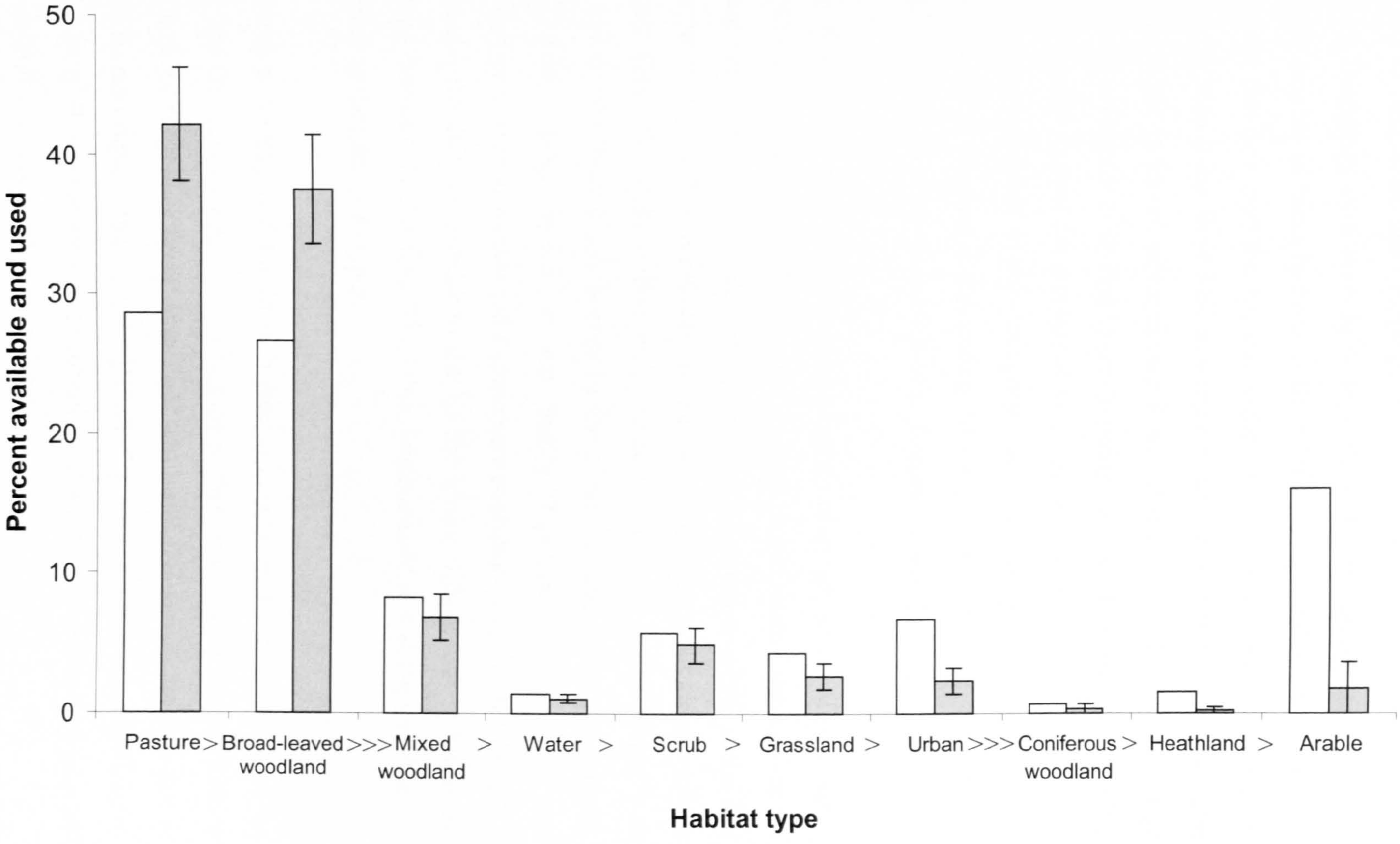


Table 4. Simplified ranking matrix of the radio-tracking data from 12 *R. ferrumequinum* comparing used foraging habitat (90% cluster cores) with the habitat available (combined MCP).

	Urban	Broad-leaved	Scrub	Grassland	Pasture	Mixed wood	Water	Arable	Coniferous	Heathland	Rank
Urban		---	---	-	---	---	---	+++	+++	+++	3
Broad-leaved	+++	+	+	+++		+++	+++	+++	+++	+++	8
Scrub	+++	-		+	---	-	-	+++	+++	+++	5
Grassland	+	---	-		---	-	-	+++	+++	+++	4
Pasture	+++	-	+++	+++		+++	+++	+++	+++	+++	9
Mixed wood	+++	---	+	+	---		+	+++	+++	+++	7
Water	+++	---	+	+	---	-		+++	+++	+++	6
Arable	---	---	---	---	---	---	---		---	-	0
Coniferous	---	---	---	---	---	---	---	+++		+	2
Heathland	---	---	---	---	---	---	---	+	-		1

Table shows habitat preference for each category on every row compared to the corresponding habitat in each column. Significant difference between the habitat types is shown by +++ (selected for) or --- (avoided) with + or – showing a non-significant trend. The ranking matrix was calculated by adding the number of + and +++ scores. A rank or 0 signifies the least preferred habitat with 9 being the most important habitat.

Figure 6. Comparison of habitat availability (combined MCP) (white bars) vs. habitat use (90% cluster cores) (grey bars) (mean % area ± SE) for *R. ferrumequinum* (n = 12). Habitat categories to the left of > are selected over those to the right with >>> showing significant difference between adjacent habitat types.



Diet analysis

A total of 1580 *R. ferrumequinum* droppings were dissected over the two study sites over three years. Droppings were unavailable from Bryanston in March, April and June 2007. The small number of bats observed in Boar Mill during the day could limit the use of the summer diet data from this site due to the low sample size (Fig. 3). However, as more bats were seen using Boar Mill as a night roost where they are able to digest food during the night (personal observation), it was decided that there was a large enough sample size of bats contributing to the droppings collected for analysis. Plotting cumulative taxa graphs against the number of faecal samples analysed showed that, on average, an asymptotic number of prey categories was reached by 12 samples at Boar Mill and 16 samples at Bryanston. Dissecting 20 samples was therefore adequate to confidently identify the species composition of *R. ferrumequinum* diet at the two sites.

The diets of the bats at both sites comprised eight orders of insects with a small percentage of unidentified remains (0.17% over 3 years). Lepidoptera (moths) were the most abundant prey type for both sites and constituted a large proportion of the diet during the summer (Fig. 7). This is reflected in the food niche breadth which was lowest in the summer (Fig. 8). Highest food niche breadth was seen in the spring with *Melolontha melolontha* (May bugs), beetles of the genus *Geotrupes* (the dor beetles), Trichoptera (caddis flies), insects in the family Tipulidae (crane flies) and Ichneumonidae (ichneumonid wasps) and Lepidoptera contributing to the diet (Fig. 7 & 8). In the autumn the diet was similar to that of the spring with beetles in the genus *Aphodius* (dung beetles), Trichoptera, Tipulidae, Ichneumonidae and Diptera (mainly dung flies) forming the main prey types.

Testing the mean percentage volume of each prey type between the two sites over the three years found that out of the nine prey types tested there was a significant difference in the % volume of *Geotrupes* (greater at Boar Mill) and Ichneumonidae (greater at Bryanston) (Wilcoxon signed ranks test, $P < 0.003$) (Fig. 9). Due to the unavailability of faecal remains from Bryanston over three months in 2007, the corresponding months' data from Boar Mill were removed from analyses.

A significant positive association between food niche breadth occurred across the two sites (Pearson product-moment correlation; $r = 0.885$, d.f. = 8, $P < 0.01$) (Fig. 8). Comparing the food niche overlap between the two sites found that there was a significant overlap in diet ($O_{jk} > 0.60$) except for October 2005 and March 2006 (Table 5).

Figure 7. Cumulative percentage volume of prey items recorded in the diet of *R. ferrumequinum* in 2005, 2006 and 2007 at (a) Boar Mill non-breeding roost and (b) Bryanston maternity colony.

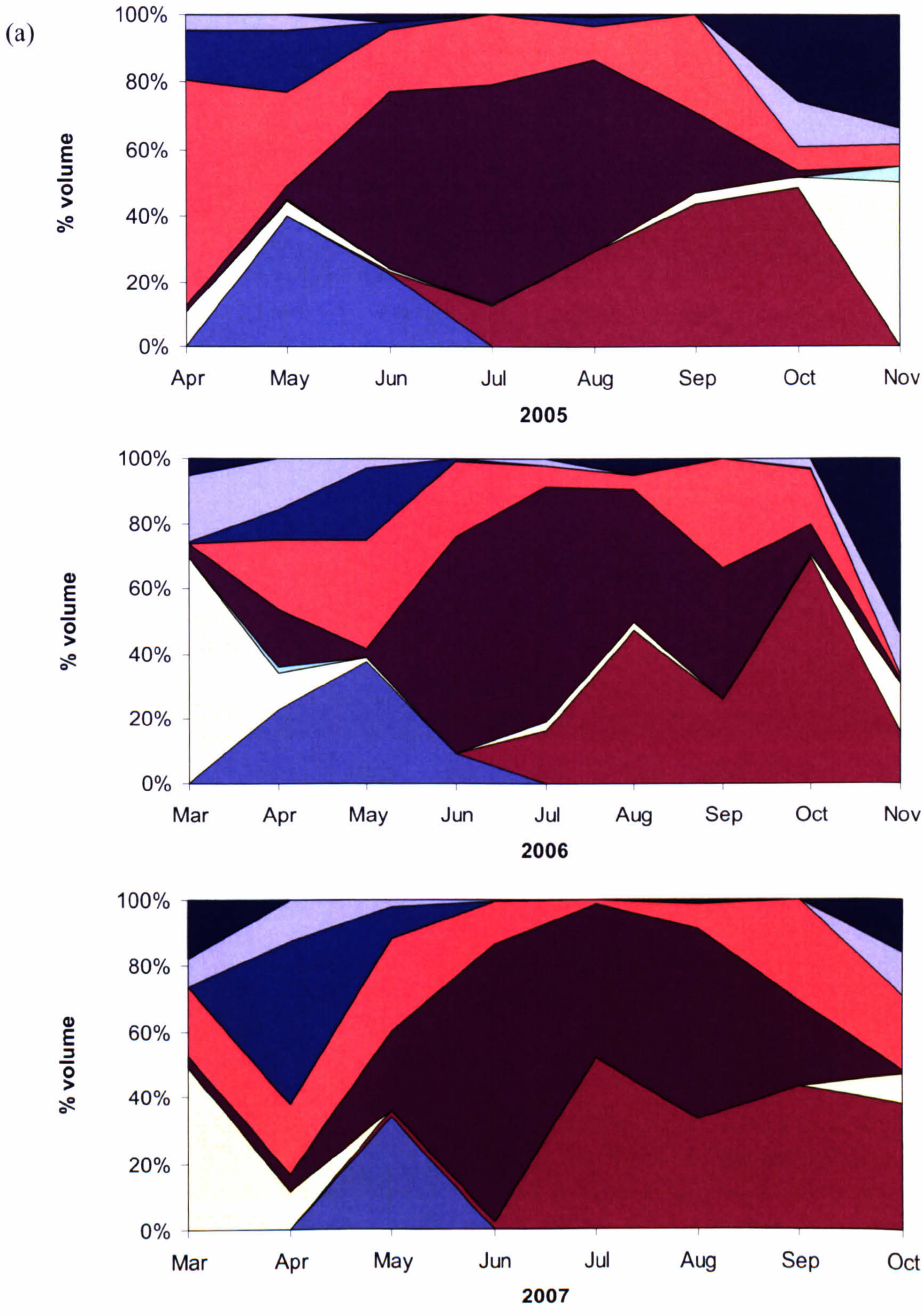


Figure 7 cont. *Data for June 2007 unavailable

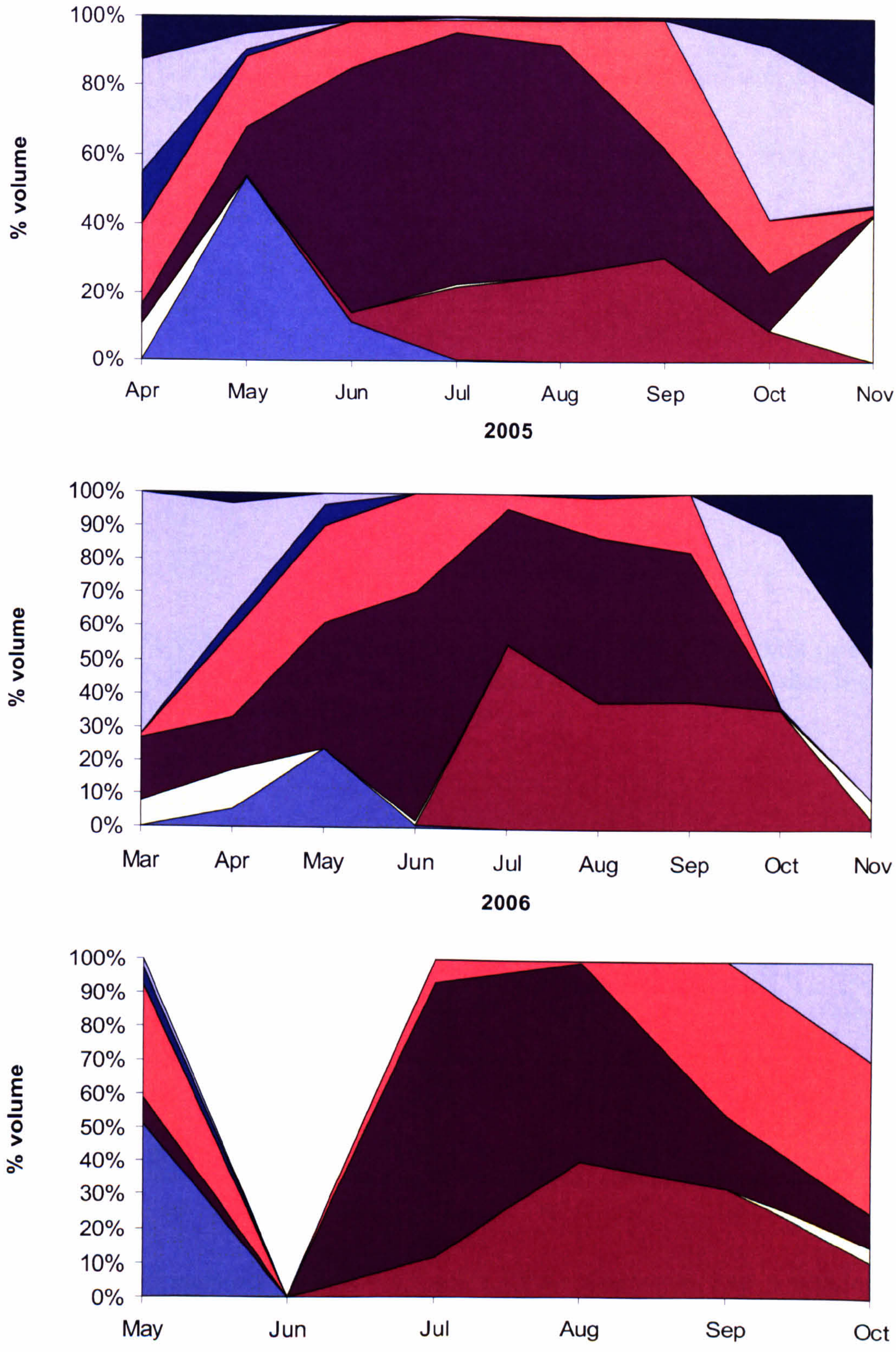


Figure 8. Comparison of mean food niche breadth of *R. ferrumequinum* diet at Boar Mill (solid black) and Bryanston (dashed) between 2005 – 2007.

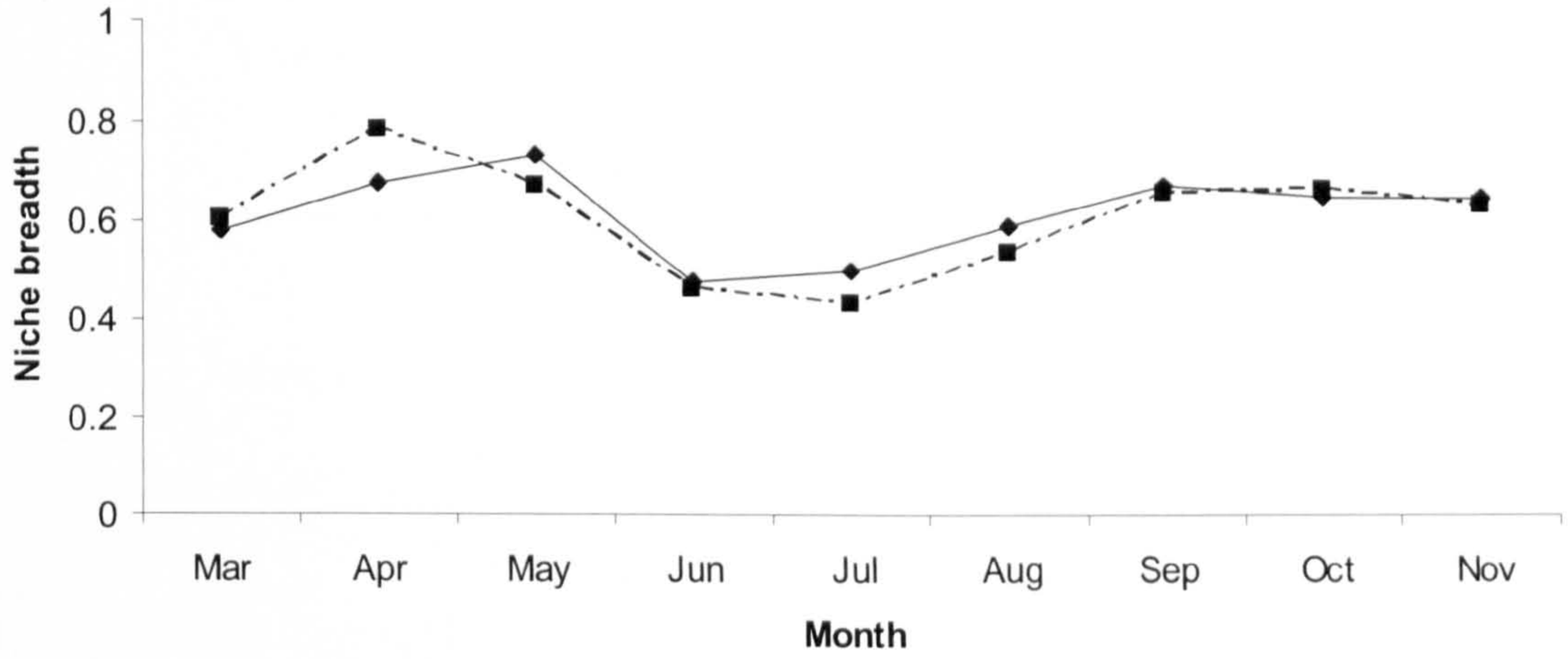
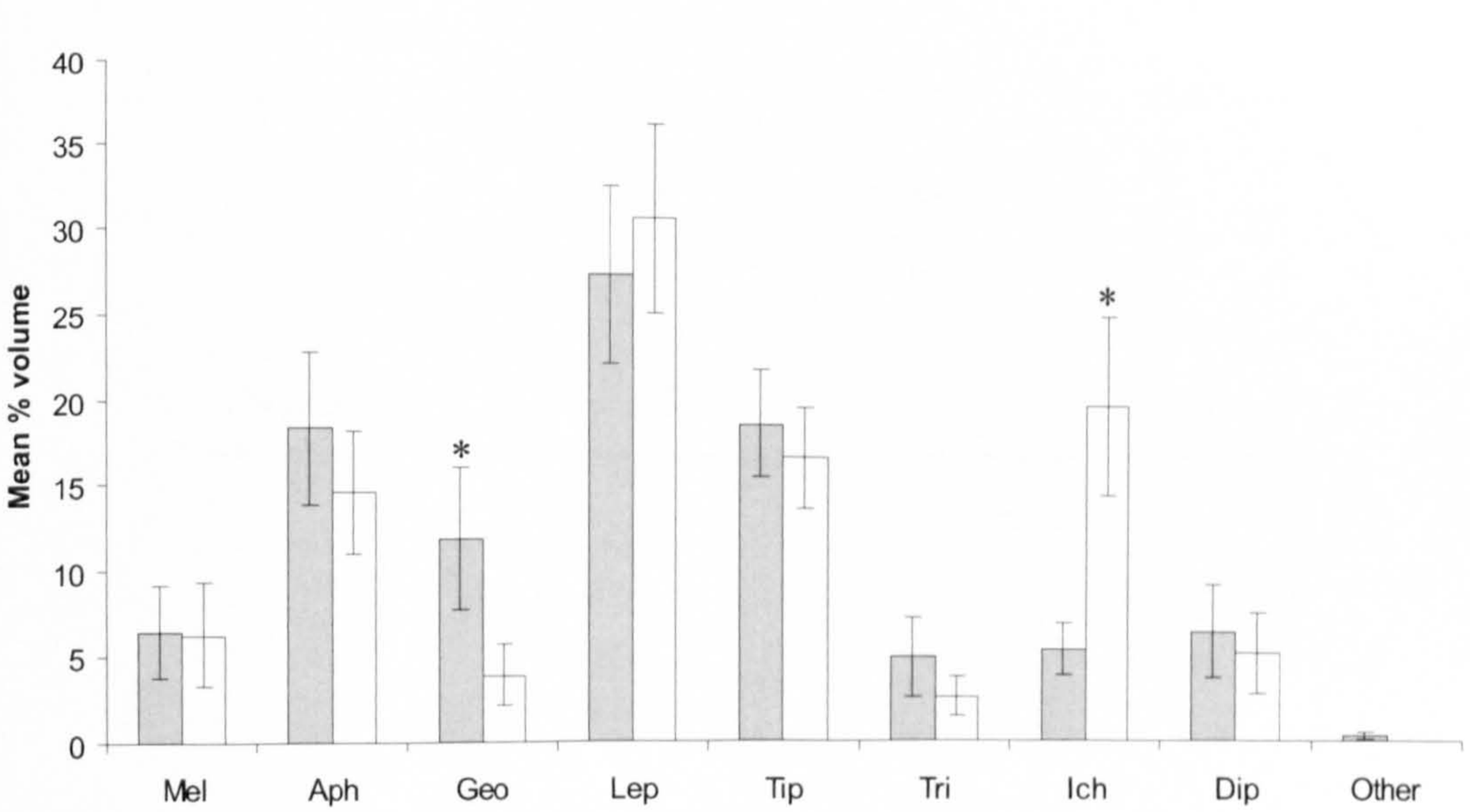


Figure 9. Mean % volume of prey identified per month (± SE) at Boar Mill (grey) and Bryanston (white) between 2005 – 2007. Significant differences between sites is shown by * ($P < 0.05$ after adjustment for multiple tests).



Mel = *Melolontha melolontha*, Aph = *Aphodius*, Geo = *Geotrupes*, Lep = Lepidoptera, Tip = Tipulidae, Tri = Trichoptera, Ich = Ichneumonidae, Dip = Diptera, Other = unidentified.

Table 5. Food niche overlap of *R. ferrumequinum* at Boar Mill and Bryanston between months and for each year. Cases where overlap was not significant ($O_{jk} < 0.60$) are shown by *.

Month	Year		
	2005	2006	2007
March	-	0.39*	-
April	0.65	0.83	-
May	0.91	0.71	0.92
June	0.97	0.99	-
July	0.96	0.75	0.77
August	0.99	0.97	0.99
September	0.96	0.94	0.95
October	0.46*	0.58	0.67
November	0.89	0.88	-
Overall	0.92	0.91	0.86

Discussion

Roost use

Through continual monitoring, this study has recorded the greatest number of *R. ferrumequinum* found using Boar Mill. It has also identified another non-breeding roost at Creech. There was a high level of roost fidelity in the spring with individuals staying at Boar Mill or Creech during the time they were being tracked. The movement of seven radio-tagged individuals from Purbeck to Bryanston and an increase in the number of ringed *R. ferrumequinum* at Bryanston (C. Morris, pers. com) indicates that this is the closest known *R. ferrumequinum* maternity roost to Purbeck. Individuals may also be using another, smaller, maternity roost ca. 16 km NE of Bryanston but access could not be obtained to confirm this. The results indicate that female *R. ferrumequinum* emerged from hibernacula in early spring and used Boar Mill and Creech as transitional roosts before moving on to their maternity colony. During this time they concentrated their foraging activity in pasture and broad-leaved woodland.

Boar Mill was used infrequently during the summer with a maximum of six bats recorded during this time. In the autumn, Boar Mill did not appear to be used by female *R. ferrumequinum* for more than two days at a time, with individuals possibly visiting mating territories at the inland quarries on the south Purbeck ridge. Between one and two adult male *R. ferrumequinum* were caught in Boar Mill during every capture session with one male being caught on three separate occasions. The presence of male bats at Boar Mill during the spring and autumn suggests that it is being used as a mating roost. Further monitoring of bat activity within the roost is needed to confirm this.

Foraging ranges and habitat use

Foraging distances from the two roosts were short (mean 2.3 km) and similar to ranges recorded in previous radio-tracking studies of this species (Jones and Rayner, 1989, Jones and Morton, 1992, Duvergé and Jones, 1994, Billington, 1999, 2000a,b, 2002, Bontadina, 2002, Billington, 2003). Habitat analysis of foraging areas revealed a high level of similarity with previous radio-tracking studies of *R. ferrumequinum* at maternity roosts (Stebbings, 1982, Duvergé and Jones, 1994, Billington, 1999, 2000a,b, Robinson

et al., 2000, Billington, 2002, Bontadina, 2002, Billington, 2003), with pasture and broad-leaved woodland selected over any other available habitat. The main difference with the results of this study compared to others carried out at maternity roosts is the frequency in which pasture and broad-leaved woodland were being used. This study found that bats spent almost equal amounts of time using pasture and broad-leaved woodland (42.1% and 37.5% respectively), supporting the findings of a previous radio-tracking study of *R. ferrumequinum* in the spring by Jones and Morton (1992) and Duvergé and Jones (1994). In the summer, *R. ferrumequinum* appears to switch foraging strategies and spend less time in broad-leaved woodland and more over pasture (Duvergé and Jones, 1994).

Switching foraging strategies between the spring and summer months is likely to be caused by changes in the climate and insect availability. The number of insects in free flight depends on population density and the level of activity, both of which are affected by climate – especially temperature (Taylor, 1963). Woodlands act as a natural temperature buffer during cold weather and can be up to several degrees warmer than the outside temperature (Langvall and Ottosson Lofvenius, 2002). These results are therefore consistent with the hypothesis that *R. ferrumequinum* is selecting habitats with the greatest availability of suitable insect prey in any given season.

Although there was some overlap of foraging areas between bats tracked during the same study period, the overlap of core foraging areas was relatively small. By only being able to radio-track a small number of individuals at any one time, it is impossible to determine whether any bats shared the same foraging area. Overlap seems unlikely however as previous studies have also found little overlap between individual foraging areas (Duvergé and Jones, 1994, Robinson et al., 2000, Billington, 2002, Bontadina, 2002, although see: Rossiter et al., 2002). A large degree of overlap between the core foraging areas of individual bats was observed over the three years of the study. Foraging site fidelity by individuals across successive years has only been shown once before (Duvergé, 1996) but, if it does occur in Purbeck, the overlap in foraging areas is unlikely to be due to bats sharing similar foraging areas at the same time. This is due to the limited time intervals in which individual *R. ferrumequinum* stay at Boar Mill or Creech before they move to the maternity roost. The gradual migration of individuals from their hibernacula to a maternity roost via Boar Mill and Creech will create unused

foraging areas and consequently reduce competition for resources. Less intraspecific competition would benefit individuals because being able to feed extensively in the spring is important for bats in order to regain the body fat lost during the winter and support foetal growth (Ransome, 1973, 1990, 2000).

Diet

The diet of *R. ferrumequinum* at Boar Mill was not significantly different from that of Bryanston. Considering the similarity in foraging behaviour between this study and others before it, this is not surprising. The main difference between the two sites was in the mean percentage volume of *Geotrupes* and Ichneumonidae consumed. The landscape between Boar Mill and Creech (where most foraging activity was identified) is dominated by pasture which may indicate a higher availability of this genus of dung beetle in this area. If *Geotrupes* are in higher abundance around Boar Mill it could explain the higher percentage of *Geotrupes* in the faecal remains compared to Bryanston. Although there is pasture surrounding Bryanston, there could be fewer beetles available per individual due to the number of bats foraging from this roost (ca. 200). An alternative explanation is that, if available, Ichneumonidae are selected over *Geotrupes* as they may be more energetically favourable (R. Ransome, pers. comm.) and found in higher abundance around Bryanston. Further dietary studies comparing the energetic costs/benefits of catching different prey items and how insect abundance relates to prey choice are needed before these questions can be resolved.

Overall, this study supports previous work showing *R. ferrumequinum* has a highly specialised diet compared to other bat species in the UK and is able to alter its foraging behaviour between the different seasons (Jones, 1990, Duvergé and Jones, 1994, Jones et al., 1995, Ransome, 2000). The similarity in diet between Boar Mill and Bryanston suggests that habitat quality may also be similar and may indicate why a maternity colony was once found at Creech. The absence of a suitable roost large enough to support a *R. ferrumequinum* maternity colony could be the limiting factor into why a colony has not established in this area.

Implications for conservation

The results of this study support previous habitat management recommendations for *R. ferrumequinum* (Duvergé and Jones, 1994, Ransome, 1996, 1997, Duvergé and Jones, 2003). Habitat selection of the 12 bats studied highlights the importance of broad-leaved woodland and pasture in the spring. If *R. ferrumequinum* non-breeding roosts are identified, management should favour enhancing broad-leaved wooded areas in close proximity of the roost as well as the availability of pasture. The results of this study suggest that management should target protecting woodland within 4 km of the roost because individuals did not tend to fly further than this when foraging. However, foraging ranges may be site specific and management recommendations should be altered accordingly if future studies show that *R. ferrumequinum* will travel further than 4 km to their foraging areas at this time of year. If a roost is being used throughout the year, management will have to consider the relative importance of pasture land and broad-leaved woodland for *R. ferrumequinum* as both habitat types are preferred at different times of the year.

The high level of variation in the number of bats using Boar Mill raises important conservation implications for the protection of non-breeding roosts. Depending on when a bat survey is carried out, very few or no bats may be seen using the roost which could lead to the importance of such a site being underestimated or even overlooked entirely. Without adequate protection, bats may be excluded from transitional roosts through the neglect or redevelopment of these sites.

Integrating new aspects of a species' life history into conservation management

Conservation biologists should aim to target aspects of a species' life history that are not currently known because without a full appreciation of its ecology and behaviour the best informed management decisions cannot be made. The majority of research on the ecology of *R. ferrumequinum* has concentrated around maternity roosts and hibernacula. This study is the first to radio-track individuals from a non-breeding roost in Dorset and provides further details on the ecology of this species at a time of year where only three studies with small sample sizes (Jones and Morton, 1992, Duvergé and Jones, 1994) have been carried out before.

Predicting species distribution of a rare woodland bat, *Myotis bechsteinii*, using knowledge-based and presence-only modelling techniques

Summary

There are relatively few examples of ecological niche models for rare or endangered species, even though rare taxa are the most important in terms of conservation management. Reasons for this can be put down to the combination of a lack of presence data associated with rare species and the uncertainty of using expert knowledge. This study evaluates the predictive power of using a knowledge-based model designed for the rare Bechstein's bat (*Myotis bechsteinii*) using information obtained from the literature before any colonies were discovered in the UK. The model was tested by comparing predicted species distributions to the locations of known maternity roosts in the south of England. The effectiveness of the knowledge-based model was then compared to a presence-only model (MaxEnt) through ground validation of an area of Dorset not previously surveyed before. Results show that when there are no data available, knowledge-based models provide an important step in highlighting areas of conservation importance. Once presence points have been identified, the use of presence-only models should be favoured as MaxEnt consistently outperformed the knowledge-based model with higher levels of sensitivity, specificity and Cohen's Kappa value of agreement.

Introduction

One of the main problems in conservation management is to determine the habitat requirements that influence crucial factors in population dynamics (i.e. births, deaths and dispersal rates) of a species (Gu and Swihart, 2003). To produce accurate recommendations, a confident description of a species' population size, distribution and habitat preferences is needed to highlight areas where research and conservation efforts should focus (Jaberg and Guisan, 2001, Agosta, 2002).

The development of geographical information systems (GIS) has allowed a more explicitly reasoned decision making process to be designed that can predict the occurrence of a species in a given area or estimate the suitability of an area for a species (Segurado and Araújo, 2004). Ecological niche models (ENM) work on the principle that there will be a range of environmental features that will influence a species' range, distribution and abundance (Yamada et al., 2003). Depending on the data available, ENMs can be calculated using empirical (e.g. generalised linear model – GLM: McCullagh and Nelder, 1989) or non-empirical methods (e.g. fuzzy knowledge-based: Salski, 1992).

ENMs can be a useful tool for large scale conservation management plans because they can predict species abundance and habitat suitability over a wide area (Guisan and Thuiller, 2005, Whittaker et al., 2005). Despite this, there are relatively few examples of ENMs for rare or endangered species, even though rare taxa are of crucial importance in terms of conservation management (Engler et al., 2004). Reasons for this include a lack of observational data available for rare species and/or poor records showing the exact locations where a species was found, leading to inaccurate positioning on a map. Another reason, and probably the most influential, is that ENMs have tended to rely on the availability of presence and absence data, at least in part, for the area being studied (for a review see: Guisan and Zimmermann, 2000). As many data sets contain presence-only data, modelling approaches have been designed to take this into account (for a review see: Tsoar et al., 2007). Presence-only models include: BIOCLIM (Busby, 1986), HABITAT (Walker and Cocks, 1991), DOMAIN (Carpenter et al., 1993),

Genetic Algorithm for Rule-set Prediction (GARP) (Stockwell and Peters, 1999), Environmental Niche Factor Analysis (ENFA) (Hirzel et al., 2002), Mahalanobis Distance (MD) (Farber and Kadmon, 2003) and Maximum Entropy Modelling (MaxEnt) (Phillips et al., 2006).

Presence/absence and presence-only models, although important, are of no use when there are no data points available for the area being studied because it is not possible to use empirically-based models to form predictive distribution maps. In order to overcome this, simple ENMs can be generated using information gained from the literature and consulting 'experts' who have had previous experience studying the species in question (Kangas and Kuusipalo, 1993, Clevenger et al., 2002, Petit et al., 2003, Rüger et al., 2005, Adriaenssens et al., 2006).

Although not formed through the standard method of multivariate analysis (Guisan and Zimmermann, 2000), 'knowledge-based' models can be clearly defined, fully reasoned decision-making processes where data can be organised and categorised according to their relative importance (Goldstein, 2000, O'Connor, 2000, Clevenger et al., 2002). Considering expert opinion may be the only information available at a given time (Pearce et al., 2001), knowledge-based approaches have proven to be an effective way to rapidly model a species' population distribution for conservation management plans before more detailed empirical models can be produced (Adriaenssens et al., 2006).

Using ENMs for rare species

Despite the availability of a number of different methods that can be used to incorporate expert opinion into predicting species' occurrence (e.g. 'rule-based methods' (Petit et al., 2003), 'multi-criteria evaluation' (Store and Jokimäki, 2003), Bayesian networks (Marcot et al., 2001) or 'fuzzy techniques' (Adriaenssens et al., 2006)) few studies use knowledge-based methods to identify the occurrence of rare species. The most likely reason is because the confidence associated with knowledge-based models may not always be high, especially for rare species, due to the limited number of variables available and the possible inconsistency between experts on the importance of some variables (Maddock and Samways, 2000). However, if the objective is to protect rare or

endangered species this may not be a major concern because it may be advisable to overestimate the area of habitat that needs to be protected (Fielding and Bell, 1997). One further problem is that an ENM cannot be used successfully unless its accuracy can be assessed (Fielding and Bell, 1997). Rare species pose considerable problems when assessing model accuracy because in many cases it is not possible to confidently identify whether a species is present or absent in a given habitat.

This study aims to determine the effectiveness of using a knowledge-based modelling technique using limited information and then compare it to a presence-only method. This will be achieved by designing a knowledge-based model for Bechstein's bat (*Myotis bechsteinii*), a rare species of woodland bat, using the information available to researchers before a number of maternity roosts were discovered. The results will then be compared to the location of known maternity roosts. A comparison of the knowledge-based and the presence-only model will be performed by measuring each model's predictive success through ground validation of an area not previously surveyed.

Assessing the effectiveness of knowledge-based and presence-only models is important because it will be able to guide future studies in creating ENMs for species where there are no data available. If successful, these models can be modified to create ENMs for other species with similar habitat requirements where information on their distribution and habitat requirements is equally limited.

Study species

Myotis bechsteinii is one of the rarest bats in Europe (Stebbings, 1988). It is classified as 'Vulnerable' by the International Union for Conservation of Nature (Hutson et al., 2001) and is listed under Appendix II of the Bonn Convention (and its Agreement on the Conservation of Bats in Europe, 1994), Appendix II of the Bern Convention (and its appropriate Recommendations) and Annexes II and IV of the EC Habitats and Species Directive. In the UK, *M. bechsteinii* is protected under Schedule 2 of the Conservation (Natural Habitats, etc.) Regulations (1994) and Schedule 5 of the Wildlife and Countryside Act (1981). Due to the difficulty in locating the roosts and hibernacula of

M. bechsteinii (Hill and Greenaway, 2005), there is relatively little information on this species' distribution and population size. Although the threats faced by *M. bechsteinii* are poorly understood, low population densities will make them especially vulnerable to habitat fragmentation and the loss of roosts and hibernacula.

By emitting relatively quiet echolocation calls that are similar to calls emitted by other *Myotis* species, *M. bechsteinii* are difficult to detect and identify confidently using conventional bat detectors (Schofield and Morris, 2000, Hill and Greenaway, 2005, Macdonald and Baker, 2005). The only way to confidently determine the presence of *M. bechsteinii* is by capturing and identifying the bat in the hand. This poses considerable problems for researchers as *M. bechsteinii* are very difficult to catch because they use forest glades and rides infrequently as flight routes, have the ability to detect very fine objects such as mist nets and usually roost deep in tree holes during the day.

Until 1998, when the first *M. bechsteinii* maternity colony was identified in the UK, only isolated individuals had ever been recorded. Technological advances over the last ten years have greatly increased capture rates of rare woodland bats through the development of a bat call synthesiser that can be used as an acoustic lure (Hill and Greenaway, 2005). Maternity colonies have been found in mature broad-leaved woodlands in Dorset, Surrey, east and west Sussex and on the Isle of Wight (Macdonald and Baker, 2005). Radio-tracking has revealed that broad-leaved woodlands with a closed canopy and well-developed understorey provides ideal foraging conditions for this species (Fitzsimons et al., 2002). This close association with a well-developed understorey will make Bechstein's bats especially vulnerable to changes in woodland management such as the restoration and creation of woodland pasture (Macdonald and Baker, 2005).

Conservation implications

Advances in technology such as time-expansion bat detectors, radio-tracking and acoustic lures, has meant an increase in records for *M. bechsteinii* in woodlands within the last ten years (Hill and Greenaway, 2005). Swarming studies have also helped assess the distribution of *M. bechsteinii* (Parsons et al., 2003a). Although important, these methods of surveying for bats are expensive and time consuming. Carrying out an exhaustive search on all of Britain's woodlands is unrealistic, so past studies have mainly focussed a number of small areas owned by conservation organisations. This process has proved effective in locating a number of *M. bechsteinii* maternity colonies, but is limited due to the non-systematic approach in surveying various woodland habitats.

By conducting extensive woodland surveys in an area that has not been covered before, a better understanding of this species' habitat requirements will be gained. This will allow for quantitative studies of habitat selection to be carried out (Walsh and Harris, 1996) so that better informed management advice can be provided in the future. By identifying new *M. bechsteinii* colonies, the trees and surrounding landscape can be protected against changes in management practice that could reduce habitat quality.

The objectives of this study are to:

1. Predict species distribution of *M. bechsteinii* maternity colonies using a knowledge-based model from data available before any colonies had been found.
2. Assess whether the knowledge-based model would have been able to accurately predict species presence in woodlands with known maternity roosts.
3. Create a presence-only model for *M. bechsteinii* using current records.
4. Measure and compare the predictive success of the knowledge-based and presence-only model through ground validation of woodlands on the Isle of Purbeck.

Methods

Study Area

Model validation was carried out on the Isle of Purbeck, Dorset. Past *M. bechsteinii* records are limited in this area because no major bat survey has been carried out in the last 30 years. Since 1947 only 22 records have been submitted to Dorset Environmental Records Centre [DERC]. The majority of these records (17) focus around the inland quarries during the autumn and winter months, a time when the bats will be swarming around the quarries or using them as hibernacula. There are a small number of records (5) of *M. bechsteinii* in areas away from the quarries but these do not include any maternity roost sites and carry low reliability ratings. Due to the age and confidence of these records they were not included in any habitat suitability model.

The locations of 24 *M. bechsteinii* maternity roosts were used in the presence-only model. These were identified in the south of England between 2000 and 2005 (Table 6).

Habitat data

Two sources of habitat data were used in the ecological niche models (ENMs). The ancient woodland inventory was downloaded from Natural England (www.naturalengland.org.uk) and Ordnance Survey (OS) MasterMap data were downloaded from Digimap (under EDINA CHEST agreement) (http://edina.ac.uk/digimap). A description of the habitat variables taken from each data source is shown below (Table 7).

Table 6. Name and location of the 24 *M. bechsteinii* maternity roosts found in the UK that were included in the presence-only model.

Place name OS grid reference	County	# records	Report
Ebernoe Common SU 980 270	West Sussex	16	Greenaway et al. (2001) plus unpublished records
Chiddingfold TQ 000 333	Surrey	3	Unpublished report
Powerstock Common SY 547 974	Dorset	2	Greenaway (2003)
Piddles Wood ST 797 127	Dorset	1	Greenaway (2003)
Melcombe Park ST 745 045	Dorset	1	Greenaway (2003)
Briddlesford Reserve SZ 549 904	Isle of Wight	1	Davidson-Watts (2002)

Table 7. Description of data source and habitat variables used in the EMNs.

Date Source	Variable	Description
Ancient woodland inventory	Ancient woodland	Land that has had continuous native or scrub cover since at least 1600 AD Has not been replanted May have been managed by coppicing or felling and allowed to regenerate naturally Over 2 hectares
OS MasterMap	Broad-leaved woodland	Dominated by semi-natural broad-leaved woodland, plantation broad-leaved woodland, coppice woodland and orchards
	Coniferous woodland	Coniferous plantations or coniferous woodland with less than 10% deciduous trees
	Mixed woodland	Woodland with over 10% deciduous and coniferous trees

Knowledge-based modelling

The knowledge-based model was designed in ArcGis 9.2 (Esri Inc.) using the ModelBuilder® extension. A 'fuzzy' approach was adopted to build the model because it provides a simple and effective method for integrating knowledge-based data (Rüger et al., 2005). In this model each variable was split into a number of categories and given a score between one and five. The resulting scores from all of the different variables are built up to produce one final output value. To standardise the output values (Cohen, 1960), all values were divided by the maximum possible score. After standardisation values range from zero (not suitable) to one (most suitable).

To prepare the GIS for running the model, variables from the ancient woodland inventory and OS Mastermap were downloaded for the south of England. Data were then converted from a vector shape file format into a grid format of 25 m x 25 m cells.

Creating the knowledge-based model

The level of habitat preference, or 'relative weightings', of each variable was determined using information from the literature published before the first *M. bechsteinii* maternity roost was discovered in 1998 (Stebbings, 1989, Harris et al., 1995, Yalden, 1999). Justification of variable selection, relative scores and model design are outlined below.

Woodland structure

Broad-leaved woodland was considered to be the most important variable (Harris et al., 1995) so given a high score. Other woodland types were seen as less important so given a lower score. Although the minimum size woodland needed to support a colony of *M. bechsteinii* is not known, larger woodlands should have a greater likelihood of supporting a colony because there should be more available resources. To determine the relative score for the size of different woodlands, the area of each woodland was calculated and five natural breaks (as calculated by ArcGIS) in the distribution drawn. Woodlands with a greater area were given a higher score (Table 8).

Woodland age

The age of a woodland may be important in predicting presence. *M. bechsteinii* is thought to roost almost exclusively in the hollows of trees (Harris et al., 1995). Mature woodlands should offer greater roosting opportunities for bats because tree cavities should be in higher abundance when compared to younger woodlands. The stable environment created by the persistence of ancient woods may increase the likelihood of discovering *M. bechsteinii* colonies. Ancient woodlands were given a higher score than other woodland types (Table 8).

Connectivity

One problem with scoring woodland size (above) is that it does not take into account how isolated woodlands are from one another. Clusters of small woodland blocks will be given a lower score than larger, more isolated woodlands. Depending on how close woodlands are from one another, they could be used by the same colony of bats and should be regarded as a single unit. To overcome this, hotspot analysis was applied to all woodlands in ArcGIS 9.2. ArcGIS calculates Getis-Ord Gi* statistics of proximity and transforms the data to output *z* scores for each woodland block. The *z* score can be negative as well as positive with a higher score revealing greater connectivity. Instead of transforming the *z* scores into a relative score between one and five they were left in their raw state because the negative values were seen useful to reduce the importance of more isolated woodland blocks (Table 8).

Model output

The series of steps to create the model, including the interaction between different variables, is shown in Figure 10. The simplest model would have been to sum all of the relative scores for each variable to produce an output map. To maximise the difference between small broad-leaved woodland (favourable) and large coniferous plantations (less favourable) this was not carried out. Instead, the relative scores for woodland size and type were multiplied against each other. To double the importance of ancient woodlands the relative scores for this variable were multiplied against the [woodland types x woodland area] output values.

Presence-only model

The model MaxEnt (version 3.1.0) (Phillips et al., 2006) was used to predict species occurrence. MaxEnt was used because it has been found to be one of the most successful ENMs when compared to both presence-only and presence-absence models (Elith et al., 2006, Hernandez et al., 2006). MaxEnt works by using a machine learning method with a maximum entropy principle to estimate species' occurrence by projecting a distribution that is closest to uniformity. This will be subject to a set of constraints that reflect the uncertain distribution of the species under each environmental variable that have been included in the model (Phillips et al., 2006).

One limitation of the current distribution of *M. bechsteinii* maternity roosts is that 16 out of 24 records are from the same woodland at Ebernoe. Due to the problems associated with pseudoreplication the model may artificially bias the importance of woodlands with similar properties to that of the woodland with 15 presence points. However, removing the pseudoreplicates from the model will reduce the sample size to 10 which may be too small for the model to perform effectively. Previous habitat modelling using MaxEnt has shown that it is robust to both small sample size and pseudoreplication (Elith et al., 2006). To confirm MaxEnts ability to perform consistently under these two constraints and provide the best *M. bechsteinii* distribution map as possible two models were produced, one using the location of 10 *M. bechsteinii* maternity roosts (pseudoreplicates removed) and the other using all 24 maternity roosts. All of the environmental variables used in the knowledge-based model (Table 7) were included in the analysis.

Habitat variables were first converted into raster format in ArcGIS 9.2. Model parameters were set to a convergence threshold of 10^{-5}, maximum number of iterations 500 and random test percentage to 0. After carrying out a number of test runs of both models the regularization parameter was adjusted to 0.1 to allow for a normal distribution of habitat weightings (Phillips et al., 2006).

Model validation

Spatial distribution maps were produced for *M. bechsteinii* using the knowledge-based and presence-only models. An arbitrary threshold of 0.7 was chosen to produce a presence-absence map for the knowledge-based model as recommended by Hirzel and Guisan (2002). A presence-absence map was drawn in MaxEnt using the logistic threshold of the 10th percentile training presence (as calculated by MaxEnt) as the cut-off value (Phillips et al., 2006).

An initial assessment of the knowledge-based model was performed by comparing the locations of the known woodlands where *M. bechsteinii* maternity roosts have been identified to the knowledge-based presence-absence map. Assessment of the predictive accuracy of both types of ENM was carried out by surveying woodlands on the Isle of Purbeck. Woodlands were picked at random so that both category types (presence or absence) from both models were surveyed.

Validation of the models was carried out in the summers of 2006 and 2007 by attracting and catching *M. bechsteinii*, under English Nature licence, in harp traps (2.4 x 1.85 m, Faunatech, Victoria, Australia) using an acoustic lure (Sussex Autobat, Hill & Greenaway, University of Sussex, UK). Trapping sessions lasted for approximately 5 hours with two harp traps used per wood that were situated between 50m – 100m apart. To limit the effect of the bats habituating to the ultrasound, the acoustic lure was used at each harp trap for 15 minutes at a time, emitting ultrasound for 3 minutes intervals with a 2 minute gap in-between. No surveys were carried out during the part of summer when the bats were thought to be giving birth (end June/beginning of July) in order to avoid causing excess disturbance to any heavily pregnant bats. The acoustic lure was used because it has been shown to be an effective survey method in attracting 'elusive species' of bat (Hill and Greenaway, 2005), minimising the effect of 'false negatives' on the results (Gu and Swihart, 2003). Presence-data was only recorded when female *M. bechsteinii* were caught because the presence of male bats does not necessarily indicate that a maternity colony is nearby. To test the effectiveness of the acoustic lure, surveys were repeated on a subset of woodlands identified in 2006 as both positive and negative for *M. bechsteinii* to see if the same results were obtained.

To positively identify *M. bechsteinii* maternity roost trees five lactating female bats were caught and fitted with radio-transmitters in August 2007. Radio-transmitters weighing 0.4 g (manufactured by Biotrack Ltd) were used on captured bats as long as they weighed less than 5% of the bats body mass (average 4.9%) (Kenward, 1992). Radio-transmitters were attached to the area between the scapulae using Skin Bond (Pfizer Inc.), a form of biodegradable glue, after clipping the bats fur. The bats roosts were located during the day using an Australis 26K scanning receiver (Titley Electronics Ltd., Ballina, Australia) with a three-element Yagi collapsible antenna (Mariner Radar, Lowestoft, UK).

Assessing model accuracy

Three measures of predictive success were used to validate the ENMs based on a presence-absence contingency table (confusion matrix) (Table 9): sensitivity, specificity and Kappa statistics (Cohen, 1960) (Equation 3, 4 and 5 respectively). Sensitivity is a measure of Type II error (predicting a species is absent when it is actually there). Sensitivity is an important measure of success because when modelling the distribution a rare species it is preferable for the model to overestimate occurrence (reducing the specificity) rather than miss areas where it is actually found. The Kappa statistic calculates the predictive success of the model after accounting for chance with indices ranging from 0.0 (no agreement) to 1.0 (spatially identical). Values of > 0.75 indicate excellent agreement beyond chance; values between 0.40 and 0.75 demonstrate fair to good agreement; and values of < 0.40 indicate poor agreement (Clevenger et al., 2002).

Table 8. Variable type and relative weighting of each category used to build the knowledge-based model. The maximum and minimum *z* scores are shown for the hotspot analysis: relative weightings were not used (see text).

Variable	Category	Relative weighting
Ancient woodland	Ancient woodland	2
	Non-ancient woodland	1
Woodland type	Broad-leaved woodland	5
	Coniferous woodland	1
	Mixed woodland	3
Woodland area (ha)	0 – 4	1
	4 – 18	2
	18 – 50	3
	50 – 155	4
	155 – 659	5
Hot spot analysis	Maximum connectivity	25
(*z* score)	Maximum isolation	-10

Figure 10. Simplified representation of the knowledge based model constructed in Modelbuilder. Blue ovals represent the initial input data, yellow squares illustrate processes and green ovals represent output data which can also be used as input data for new processes. All ovals are labelled to indicate data type and process action.

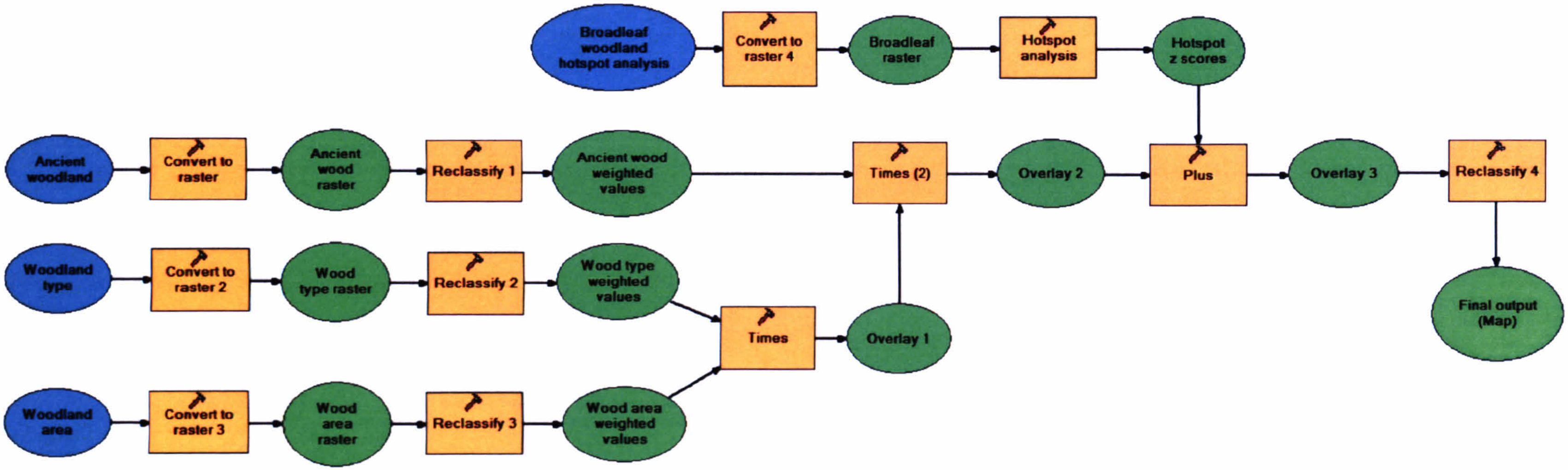


Table 9. Illustration of a presence-absence contingency table used to calculate sensitivity, specificity and Kappa (Equation 3, 4 & 5 respectively).

	Measured		
Predicted	Present	Absent	Total
Present	A	B	A+B
Absent	C	D	C+D
Total	A+C	B+D	A+B+C+D

Where A = true positive, B = false positive, C = false negative and D = true negative

Equation 3. Sensitivity

$$\text{Sensitivity} = \frac{A}{(A+C)}$$

Equation 4. Specificity

$$\text{Specificity} = \frac{D}{(D+B)}$$

Equation 5. Kappa

$$\text{Kappa} = \frac{(A+D)(A+B+C+D) - (A+B)(A+C) - (C+D)(B+D)}{(A+B+C+D)^2 - (A+B)(A+C) - (C+D)(B+D)}$$

Results

Knowledge-based model accuracy to predict current *M. bechsteinii* roosts

The knowledge-based model correctly predicted 6 out of the 10 woodlands where known *M. bechsteinii* maternity roosts have been found across the south of England. Using this method woodland containing 20 out of the 24 maternity roost trees would have been identified (Fig. 11). Calculating the sensitivity of the knowledge-based model in predicting the occurrence of maternity roosts in these woodlands (0.60) revealed that there was a 40% chance of the model predicting *M. bechsteinii* was absent from a wood when it is actually there (Type II error). Although the type II error is relatively high, further analysis of the habitat prediction map at Powerstock showed that the maternity roost was less than 500 m away from woodland highlighted as 'suitable' by the model and both of the roosts at Chiddingfold were between 400 – 800 m of 'suitable' habitat (Fig. 11). Identification of these roosts through surveying woodlands nearby would have meant that the knowledge-based model would have helped identify the woods containing 23 out of the 24 maternity roosts.

Validation and comparison of the knowledge-based and presence-only models

A total of 43 woodlands were surveyed on the Isle of Purbeck between 2006 – 2007. During this time ten female *M. bechsteinii* were caught in five different woodlands. Radio-tracking in 2007 identified three maternity roost trees that appear to belong to two separate colonies. One colony was found using two of the identified trees located in the north of Purbeck at Goathorn (ca. 35 individuals using each tree) and the other colony using a single tree to the south at Langton (ca. 90 individuals) (Fig. 12). With the exception of the maternity colony at Goathorn using one oak tree which was very small (diameter at breast height [DBH] < 1m), these maternity roost trees are structurally similar to previous trees identified in the south of England (DBH > 1m) (Flanders, 2005). During the period in which individuals were being tracked there was no movement between the two colonies.

Identical presence-absence data was obtained in 2007 when surveying a subset of 12 woodlands identified in 2006 as being positive (n = 4) or negative (n = 8) for *M.*

bechsteinii. The acoustic lure has proved to be an accurate method for identifying presence and absence data for this species (Hill and Greenaway, 2005).

The output maps produced by MaxEnt using both sets of input data (10 and 24 presence points for woods and roosts respectively) produced identical results. These results support the findings of Elith et al. (2006) in showing that MaxEnt is robust when processing both small sample sizes and pseudoreplication because the same predictions resulted from the use of woods or roosts within woods during data input.

Testing the predictive accuracy of both models found that the MaxEnt model outperformed the knowledge-based model in all three measures of model success (Table 10). A Kappa value of 0.15 for the knowledge-based model indicates poor predictive performance (Clevenger et al., 2002) while the value of 0.55 for MaxEnt shows fair to good agreement (0.40 – 0.75). Although both models were unable to predict the *M. bechsteinii* colony using the two trees at Goathorn (Fig. 12) MaxEnt outperformed the knowledge-based model because it was statistically more sensitive producing a greater number of true positive predictions.

Figure 11. Habitat distribution maps created by the knowledge-based model for *M. bechsteinii* in the four regions where maternity roosts have already been identified (green dots) (n = 24). Black areas show woodlands predicted to support *M. bechsteinii* colonies and grey areas represent less suitable woodland types. Four areas represent (a) Piddles wood (north) and Melcombe Park (south), (b) Powerstock Common, (c) Briddlesford and (d) Chiddingfold (north) and Ebernoe Common (south).

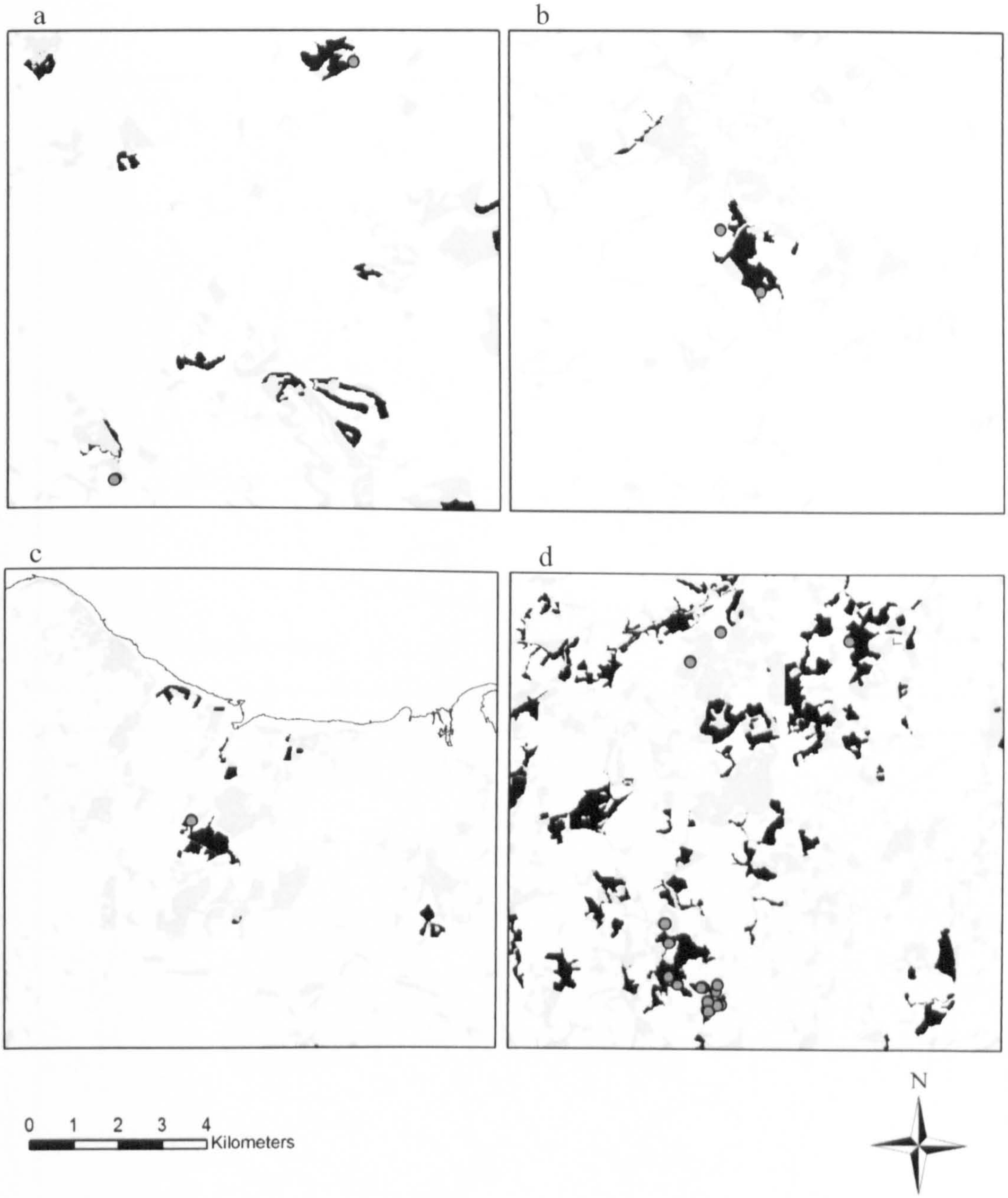
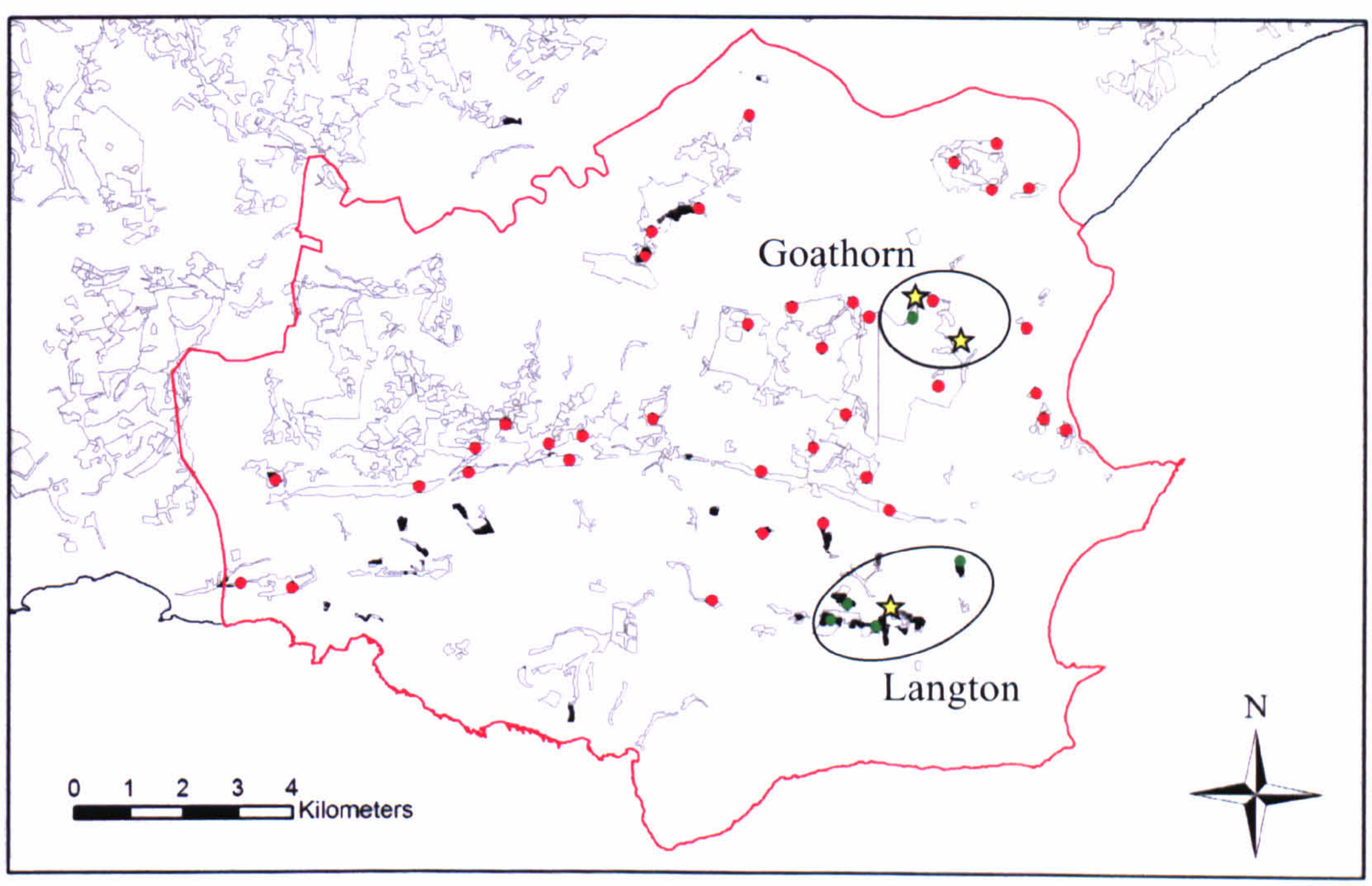


Figure 12. Ground validation of predictive maps for the distribution of *M. bechsteinii* on the Isle of Purbeck using (a) presence-only model (MaxEnt) and (b) knowledge-based model. Black areas show woodlands predicted to support *M. bechsteinii* colonies and grey areas represent woodland predicted to support absence. Absence data (red circles), presence data (green circles) and maternity colonies (yellow stars) are also shown.

(a)



(b)

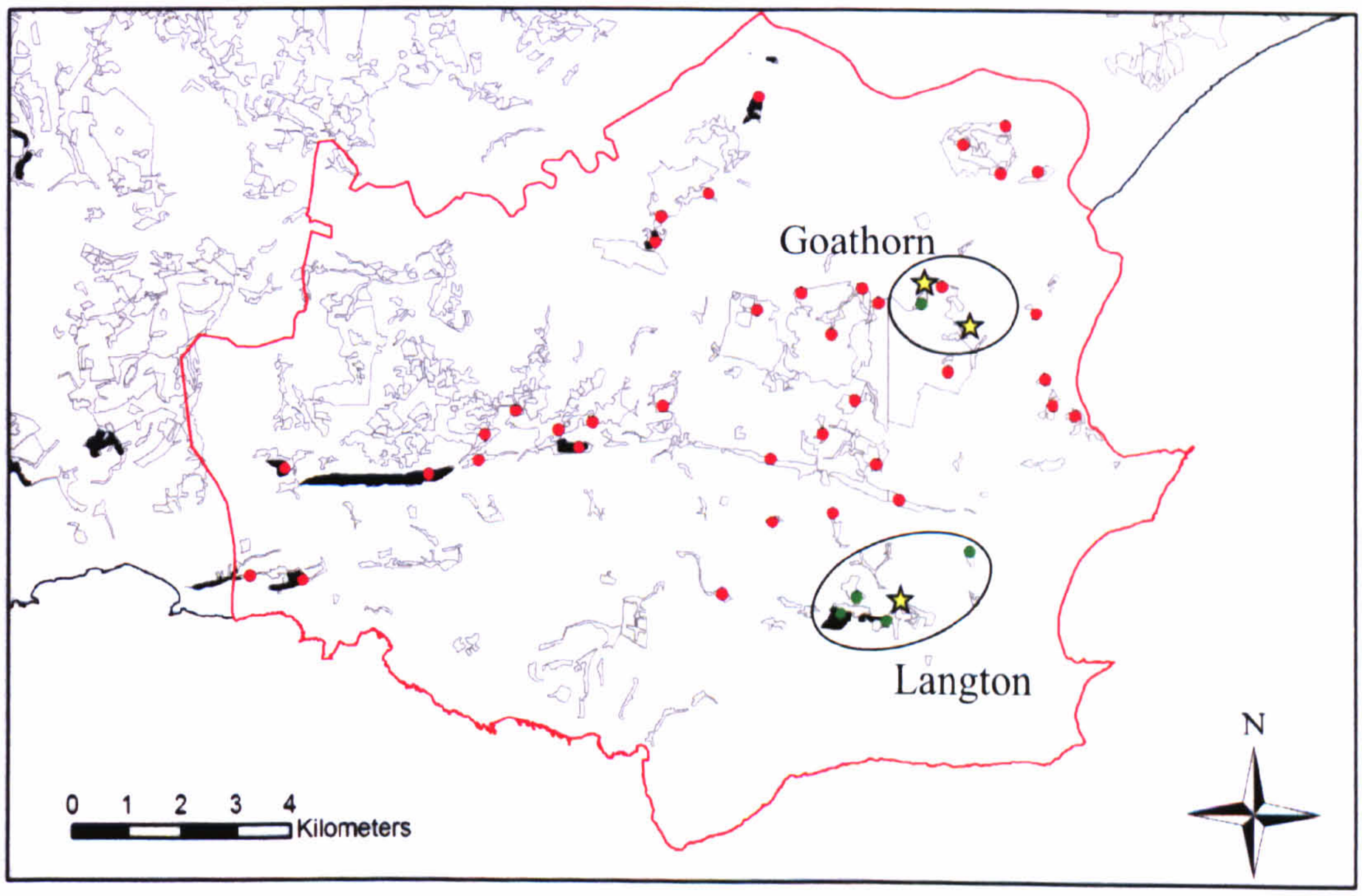


Table 10. Performance measures of the knowledge-based and presence-only models used to predict the occurrence of *M. bechsteinii* maternity colonies on the Isle of Purbeck. Total number of woodlands surveyed (n) = 43, total number of presence points = 6 (including presence point of maternity roost at Langton).

	Model	
Performance measures	Knowledge-based	Presence only (MaxEnt)
True positive (A)	2	5
False positive (B)	6	5
False negative (C)	4	1
True negative (D)	31	32
Sensitivity	0.33	0.83
Specificity	0.84	0.86
Kappa	0.15	0.55

Discussion

Although the use of ENMs is widespread there are still relatively few examples of models created for rare or endangered species. Reasons for this are thought to include the lack of data available for empirical models to use and the inability to accurately validate a model's accuracy (Engler et al., 2004). A knowledge-based modelling approach is often seen as too subjective to be reliably used (Maddock and Samways, 2000). However, this study has shown that the knowledge-based model would have been an effective method in highlighting woodlands supporting *M. bechsteinii* maternity colonies when no other data were available. The knowledge-based model was not always able to predict the exact locations where maternity roost trees were found but, with the exception of Melcombe Park, it identified concentrated areas of suitable habitat close to roost sites. If *M. bechsteinii* surveys had been carried out in woodlands identified by the model at both Powerstock and Chiddingfold it is likely that foraging individuals from these maternity roosts would have been caught and radio-tracked back to these roosts.

Ground validation shows the importance of carrying out field surveys to assess model accuracy because there is little point designing an ENM if its accuracy cannot be assessed. Confidence is needed in ENMs because management decisions cannot be made using results that are potentially subject to high levels of error (Fielding and Bell, 1997). Model accuracy is also important because it may be acceptable to over-estimate presence (false positives) when managing for rare or endangered species (Fielding and Bell, 1997). In this study model accuracy was very low for the knowledge-based model although MaxEnt showed a fair to good level of agreement with a Kappa value of 0.55 (Clevenger et al., 2002). Considering *M. bechsteinii* is a rare species and only a limited number of habitat variables were used, model accuracy should improve as more roosts are identified and its ecological requirements are better understood. One problem facing the development of future ENMs for *M. bechsteinii* is the availability of relevant habitat data. With the exception of the small oak tree used as a roost at Goathorn, the results of this study support other findings that *M. bechsteinii* is highly dependent on mature trees with a large diameter at breast height (DBH) and which support one or more

woodpecker holes (Flanders, 2005). Obtaining specific habitat variables such as these across the whole of England would be extremely difficult because it would require individual woodland surveys to be carried out. Surveys of this nature would be extremely time consuming and expensive and is a likely reason why this type of data is not already available.

Neither ENM used in this study was able to predict the location of the two maternity roost trees at Goathorn and the knowledge-based model did not identify five maternity roosts found in other parts of the south of England. The identification of these false negatives is concerning because it means that if management advice had been based exclusively from the information from one of these models suitable *M. bechsteinii* habitat could have been lost. The *M. bechsteinii* colony at Goathorn represents an anomaly according to previous knowledge of this species' roosting behaviour (Stebbings, 1989, Harris et al., 1995). One of the roosts was found within a small strip of oak trees (*Quercus* sp.) bordering a coniferous plantation and the second roost was found in an oak tree marking out an ancient field boundary. Although further research is needed, it is possible that this is a relic population surviving on small strips of broad-leaved woodland due to the conversion of large areas of broad-leaved woodland into coniferous plantations. If correct, it is not surprising that neither model was able to predict the occurrence of this population and shows that less suitable areas cannot be dismissed entirely and should be checked when resources are available. Assessment of the two models in this study would therefore suggest that *M. bechsteinii* may show some flexibility in maternity roost location as long as it is in close proximity to core foraging areas.

Radio-tracking *M. bechsteinii* individuals proved vital in identifying all three of the maternity roosts and illustrates the need to integrate different methods to maximise the information obtained. Effective conservation management involves the protection of both roosting and foraging sites (Jaberg and Guisan, 2001, Agosta, 2002) and cannot be achieved using the ENMs alone.

Model reliability can be greatly influenced by small sample sizes and location errors of the presence data (Guisan et al., 2007). The use of the acoustic lure overcomes these problems as it is an effective way of providing more refined habitat data while radio-tracking individuals can identify maternity roost trees. Creating and validating ENMs for rare species is problematic because only a small number of presence points are found even when a large number of surveys are carried out. The predictive power of MaxEnt has been shown to be robust to both small sample size and pseudoreplication. To improve the model accuracy so that a Kappa value greater than 0.55 is achieved more areas need to be searched so a greater number of colonies in different woodlands can be identified.

Comparing the predictive success of the knowledge-based model to MaxEnt shows that presence-only models should be used whenever there are enough data available. MaxEnt proved to be more reliable than the knowledge-based model during validation across all three performance indicators, showing that there are limitations in using expert knowledge alone. Compared to designing a knowledge-based model, MaxEnt was easy to use, simple to interpret and took away any subjectivity in deciding the levels of habitat suitability. Using the knowledge of experts has been proposed as a way of improving the reliability of empirically based models (Yamada et al., 2003, Rüger et al., 2005). This could be important for presence-only models because without any true absence data there may be an unknown sampling bias caused by a non-systematic approach to data collection (Sattler et al., 2007). Although this may be true for some species, the apparent dependence of *M. bechsteinii* on mature broad-leaved woodland did not limit the reliability of MaxEnt despite all of the records being obtained from surveys carried out in conservation areas largely supporting ancient or mature broad-leaved woodland.

Conservation implications

Predicting species distributions is an important tool in conservation management. ENMs greatly reduce the logistical and financial problems associated with identifying species presence caused by the non-systematic approach to surveying. Knowledge-based models are important because they provide the first step in this process by creating

presence/absence data when there is little or no information available. ENMs should be continually developed so that the confidence in their predictive power is as high as possible. Improvements should include the inclusion accurate presence and absence records and the inclusion of as many habitat variables that are available. Finally, a predictive habitat model cannot be used successfully unless its accuracy can be assessed (Fielding and Bell, 1997). Designing ENMs for rare species can pose considerable problems as model validation may not be possible. The inability to assess an ENM is likely to be a key reason why relatively few studies have focussed on rare species in the past. The development of new techniques to survey rare species, such as the acoustic lure for some species of bats (Hill and Greenaway, 2005), is important so that ENMs can be used effectively.

The two new *M. bechsteinii* populations discovered on the Isle of Purbeck will now have an increased level of protection that will ensure this rare species of bat can be conserved in this region. Future work should continue surveying new areas for *M. bechsteinii* using modelling techniques such as MaxEnt so that more presence-absence data are obtained and models can be refined and enhanced. Greater confidence in the ENMs for this species will allow for large scale assessments across the south of England to identify potential areas where isolated *M. bechsteinii* populations may be found. By enhancing the habitat around these areas there is a greater potential for migration events and the colonisation of new areas.

Investigating the importance of underground sites and the impact of disturbance for swarming bats in Purbeck

Summary

Underground sites perform an important role in the autumn when a number of species of temperate zone vespertilionids visit them in an event described as autumnal swarming. This study compares bat activity at four quarries (two recently restored, two original/abandoned) surveyed on the same night and located within 400 meters of one another over three years. The swarming activity at these sites was then compared to a coastal mine 3.1 km away. A total of 838 bats of 8 species were caught over 64 capture occasions. High levels of variation in abundance of different species was observed within and between different quarries indicating that there are multiple factors governing site selection by swarming populations. Compared to the quarries, the coastal mine had greater species richness and abundance.

This is one of the first studies to compare the level of bat activity at a number of structurally different quarries in a small area. The large number of bats caught over the three years highlights the importance of underground sites for bats. High levels of variation between quarries outlines the need for a better understanding of the factors influencing a site's suitability for the bat communities using it. Finally, these results show the importance of surveying all of the underground sites within an area to assess their significance for different species of bat.

Introduction

The importance of underground sites for bats

Natural caves and man-made structures such as mines and quarries provide some of the most important roosting and mating sites for bats in the world (Racey and Entwistle, 2003). An underground site's structural longevity and stable thermodynamic characteristics can make it suitable for a wide range of bat species throughout the year (Altringham, 1996) and the availability of such sites may limit a species' population size and distribution (Kunz, 1982). In some parts of the world the number of bats roosting in a single cave system can range up to hundreds of thousands (e.g. the bent-winged bat *Miniopterus schreibersii* in Australia, Dwyer and Hamilton-Smith, 1965) to even millions (e.g. the Mexican free-tailed bat *Tadarida brasiliensis* in the United States, Davis et al., 1962). Cave roosts may also comprise of a few to many different species (Bateman and Vaughan, 1974). In North America, Pierson (1998) found that 21 of the 45 bat species regularly use underground sites with many others occasionally visiting them. Bats are often faithful to one or a few underground sites during their lifetime and can travel large distances to reach them (> 100 km) as shown by radio-tracking, ringing and genetic analysis studying migration patterns of summer roosting bats and the distribution of bats at their hibernacula (Davis and Hitchcock, 1965, Griffin, 1970, Altringham, 1996, Petit and Mayer, 2000, Fleming and Eby, 2003).

The structure of an underground site is important as it will influence variables such as temperature, humidity and cavity size which are all important factors in determining the number of species, and individuals, that can use a single site (Tuttle and Stevenson, 1978). The most commonly used sites often have more than one entrance, a degree of air flow running through them and a number of areas with different thermal properties (Tuttle and Stevenson, 1978, Altringham, 1996). Sites with only one entrance may have very little temperature variability due to the limited air flow running through them. This can make them too cold to be used as summer maternity roosts and sub-optimal during the winter as different species will have specific microclimate requirements that may change throughout the winter (Tuttle, 1979).

In addition to providing shelter for bats, underground sites perform an important role in the autumn when a number of species of temperate zone vespertilionids visit them in an event described as 'autumnal swarming' (Fenton, 1969). Swarming has been described in North America (Davis and Hitchcock, 1965, Hall and Brenner, 1968, Fenton, 1969, Humphrey and Cope, 1976, Thomas and Fenton, 1979), continental Europe (Bauerova and Zima, 1988, Degn et al., 1995, Furmankiewicz and Altringham, 2007) and more recently in the United Kingdom (Parsons and Jones, 2003, Parsons et al., 2003a, Parsons et al., 2003b, Pugh and Altringham, 2005, Rivers et al., 2005, Rivers et al., 2006). Swarming involves large numbers of bats visiting a site throughout the night, for what may only be a few hours for any one individual, before leaving again. Ringing studies have shown that on a nightly basis there is a high turnover of individuals with very few bats remaining at the site during the day (Parsons et al., 2003b, Rivers et al., 2006). After travelling to these sites from a large catchment area, bats rarely return to the same swarming site during the same year (Parsons and Jones, 2003, Rivers et al., 2006). Populations of bats at swarming sites are heavily male-biased (Parsons and Jones, 2003). Swarming is most prevalent among bats in the genus *Myotis*, but has also been found in other species and genera of microchiropteran bats (Stebbings, 1988, McDonald and Tattersall, 2001, Furmankiewicz and Altringham, 2007).

There is still a lot to be discovered about the function of swarming, but the most widely supported explanation through behavioural (Fenton, 1969, Thomas and Fenton, 1979) and genetic (Kerth et al., 2003, Veith et al., 2004, Rivers et al., 2005, Furmankiewicz and Altringham, 2007) studies is that it is a mating event. Swarming results in outbreeding and consequently gene flow among bat populations that are often highly philopatric, and consequently isolated, during the summer (Parsons and Jones, 2003, Rivers et al., 2005). Other suggested functions for swarming include assessment of suitable hibernacula (Fenton, 1969), information transfer of hibernation sites from adult to juvenile bats (Davis and Hitchcock, 1965, Fenton, 1969, Cope and Humphrey, 1977), use of 'intermediate' roosts on migration routes between summer and winter regions (Whitaker, 1998) or a combination of the above (Bradbury et al., 1986).

Assessing the importance of underground sites for bats

The majority of swarming studies carried out to date have focussed on describing the bat activity at individual sites. When multiple sites have been surveyed they have been carried out on separate nights due to the impracticality of moving between them (Parsons et al., 2003a, Rivers et al., 2006). Large night to night variation can be seen in the number of bats caught at swarming sites (Humphrey and Cope, 1976), and may be partly explained by changes in the weather (Fenton, 1969, Parsons et al., 2003b). The inability to compare different underground sites within the same area has meant that their relative importance to different bat communities using them has not been assessed. This is unfortunate because the level of bat activity by swarming communities has been found to be highly correlated to the height and width ratio of mine entrances, with taller and narrower entrances attracting the greatest number of species and individuals when compared to shorter and wider sites (Johnson et al., 2006, although see: Glover and Altringham, 2008). Previous work that has combined the results taken from swarming surveys of different sites carried out in the same area could be misleading if the sites are structurally different and may overlook the importance of some sites over others (Parsons et al., 2003a, Rivers et al., 2006).

The following study aims to address this issue by assessing the importance of a number of structurally different quarries on the swarming bat communities and *R. ferrumequinum* population found on the Isle of Purbeck, Dorset. By catching bats on the same night at a number of unrestored and renovated quarries in a small geographical area this study will compare the level of activity and species composition between the different sites to determine whether there is any difference between them.

Monitoring underground sites

Although highly intrusive and resource demanding, catching bats as they enter or exit an underground site is the only method that can be used to describe the species composition, age and sex ratio of bats caught throughout the night. Catching bats has provided a number of studies the opportunity to mark and radio-track individuals in order to estimate the number of bats using a swarming site per night, how much movement there is between different sites and over what distances individuals travel to

get to them (Parsons et al., 2003b, Rivers et al., 2006). This form of survey can be particularly useful when monitoring the species composition of bats leaving their hibernation sites in early spring as many crevice dwelling species will be missed during hibernation counts (Hall and Brenner, 1968, Stebbings, 1988, Degn et al., 1995, Parsons et al., 2003a, Rivers et al., 2006).

The objectives of this study are:

1. To investigate the level of swarming at different quarries in Purbeck.
2. To record the species composition, abundance, age and sex ratios at the different quarries over time.
3. Compare the swarming activity in Purbeck with previous studies carried out.
4. Assess whether the impact of restoring quarries has a negative effect on the swarming bat communities and *R. ferrumequinum* population using them.
5. Provide recommendations on the conservation and long term management of swarming sites for bats.

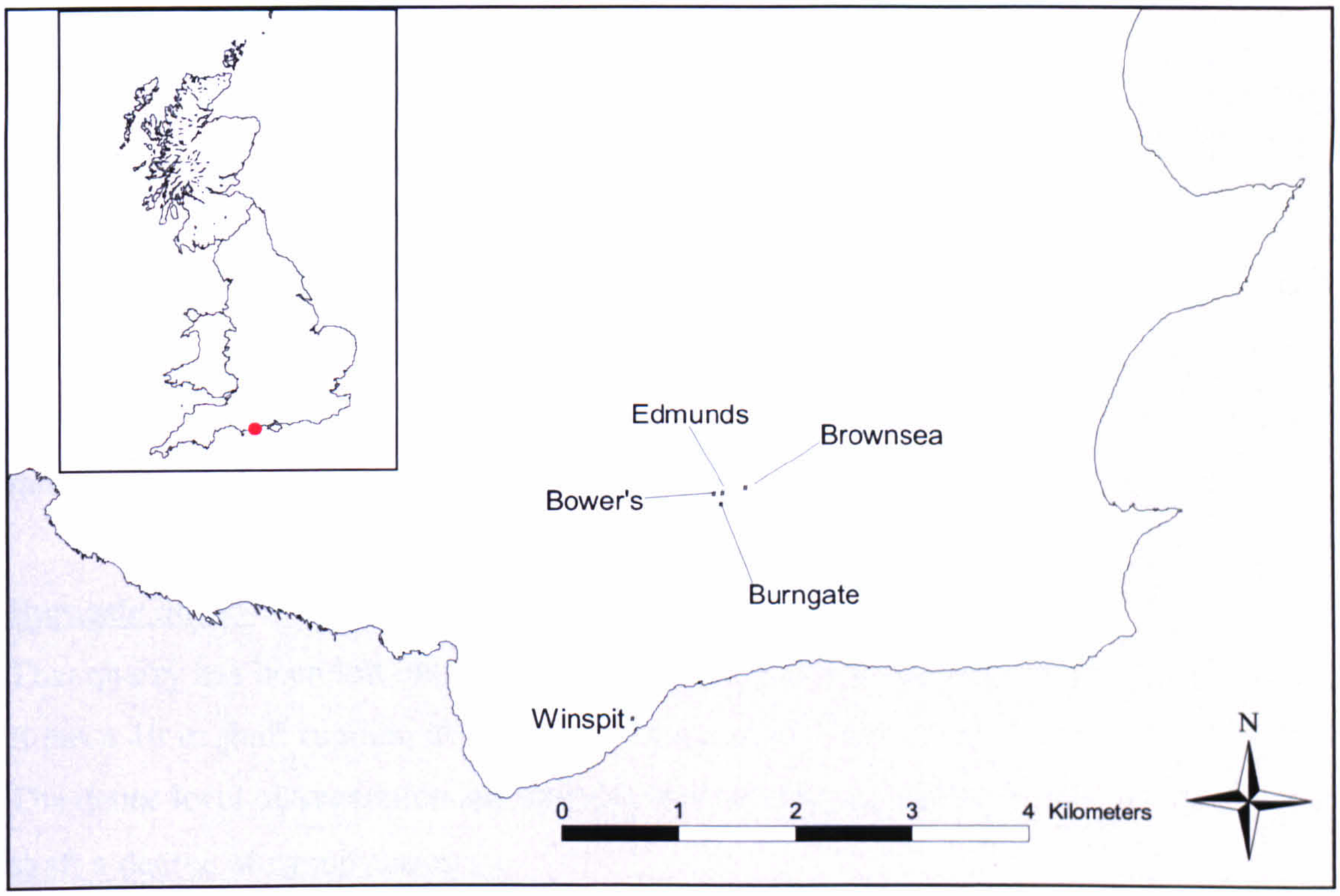
Methods

Study sites

This is the first study to measure swarming activity of bats on the Isle of Purbeck, Dorset. It was carried out at four inland quarries (2 restored and gated, 2 original/unrestored and not gated) at Acton and one coastal mine (gated) at Winspit (Fig. 13 & 14). All of the quarries at Acton are within 400 m of one another and are approximately 3.1 km from the mine at Winspit. Before access into these sites was forbidden under current National Trust health and safety guidelines, previous surveys found that all sites were used by *R. ferrumequinum* throughout the year with highest numbers recorded during winter hibernation counts (Stebbings, 2003). Hibernation counts made between 1948 – 2003 identified 11 species of vespertilionid using the quarries (Stebbings, 2003) but these records are limited in use as they are not site-specific and do not identify the number of individuals found. Due to the close proximity of all of these sites it is reasonable to assume that the quarries chosen for this study have been, or continue to be used by many of the species identified by the hibernation checks.

The sites identified below were selected as they represent different structural properties (see below) and landscape features due to the way in which they were originally created. Moreover, restoration work has been carried out on two of the sites. Traditional inland quarries were created by digging inclined shafts down into the ground to extract the stone from beneath the layers of soil and clay while coastal mines were made by cutting straight into the side of a cliff and extracting the stone on the same horizontal plane at which the digging began.

Figure 13. Map of swarming sites on the Isle of Purbeck. Map of the UK (inset) illustrates the location of the Isle of Purbeck in context to the rest of the UK (red dot).



Acton

Since inland quarrying was replaced by open cast methods in the early 1900's, over 70% of the quarries in this area have collapsed. Between 1995 – 2000 the National Trust restored the entrances of four quarries at Acton (Brownsea Bower's, Norman's, Edmunds east and Edmunds west) with the aim of protecting these sites for bats and making the area safe for members of the public. Restoration involved clearing the site of any surrounding vegetation so that a mechanical digger could remove the earth surrounding the entrances allowing a thick lining of concrete to be laid. A gate was fitted at entrance to the tunnels before local stone was used to hide the concrete and make each site look aesthetically pleasing.

Burngate quarry

This quarry has been left untouched since it was last worked in the early to mid 1900's. It has a 10 m shaft running down at approximately 45 degrees to three tunnel entrances. The dense level of vegetation surrounding this site also provides the quarry entrance and shaft a degree of canopy cover.

Ivamy Bowers and Sheffy Harris' quarry

This quarry (hereafter referred to as 'Bowers') has been left untouched since it was last worked in the early 1900's. It has a shorter and steeper shaft that Burngate quarry and has a higher level of vegetation overhanging the shaft. There is also a dense level of vegetation surrounding the site.

Edmund's west quarry

The entrance and surrounding walls to Edmund's west quarry (hereafter referred to as 'Edmunds') were restored between 1997 – 1998. The entrance to this site is more open than that of the two unrestored quarries as the earth was not fully built up either side of the shaft once the concrete walls were in place. Apart from bramble encroaching down the shaft entrance, there is very little vegetation surrounding the site due to livestock grazing up to the quarry wall. When installing the gate at the quarry entrance a layer of concrete was built up around all four sides of the existing hole so that the gate could be supported.

Brownsea Bower's quarry

This quarry (hereafter referred to as 'Brownsea') was restored between 1997 – 1998 using a different technique to that of Edmunds quarry as the quarry entrance is located in a rectangular pit measuring approximately 10 m x 12 m x 8 m (length x width x depth). The walls to the pit were reinforced with concrete and a gate fitted to the quarry entrance. The level of vegetation around this site is greater than that of Edmunds quarry due to the build up of vegetation within the pit as it is inaccessible to any livestock. A number of trees growing near the top of the wall were left untouched and now overhang the quarry entrance.

Winspit

Port Arthur mine

This sea-cliff mine (hereafter referred to as 'Winspit') was gated in the 1980's (exact date not known) to protect bats from disturbance and to prevent the entrance from collapsing. Although once part of a more extensive tunnel system with multiple entrances along the cliff face, due to the collapse of a number of tunnel systems, Winspit has now only got the one gated entrance/exit point. The walls surrounding this site are approximately 20 m high with a gap of approximately 8 m providing the main path to access the site. The vegetation cover reduces this path to approximately 1 m wide but then clears before the entrance to the quarry.

Figure 14. Characteristics of the three quarry types used in the swarming survey. (a) abandoned, unrestored inland quarry, (b) restored inland quarry and (c) coastal mine.

(a)

View at ground level of the shaft entrance at Burngate quarry

View looking down the shaft entrance at Ivamy Bowers and Sheffy Harris' quarry

Figure 14. Cont.

(b)

View at ground level of Edmund's west quarry

View looking down the shaft entrance at Edmund's west quarry

Figure 14. Cont.

(c)

View of Winspit sea-cliff mine from ground level

Capture and processing of bats

The catching of bats was only carried out during the main swarming season (August – October) in order to limit disturbance. At other times of the year passive monitoring was carried out to assess the level of bat activity at the Acton quarries using Anabat II remote recording systems (Titley Electronics, Ballina, Australia). This was carried out for one night at the beginning and middle of each month between March and July and every two weeks in August. Anabat recorders were not left out for longer periods of time as their security during the day could not be guaranteed. Passive monitoring was carried out throughout the spring and summer as it enabled swarming catches to begin at the start of the swarming season as indicated by an increased level of bat activity at the sites.

Bats were caught using one (at all Acton quarries) or two harp-traps (Winspit) (2.4 x 1.85m, Faunatech, Victoria, Australia) that were placed to cover the entrances to the individual sites. In order to minimise the level of disturbance to the bats, catches were made no earlier than once every two weeks at any one site and traps were erected in order to maximise the number of bats caught without completely enclosing the entrance. Traps were placed at similar locations at each site for approximately 1 hour before sunset and remained up for a minimum of 5 hours and a maximum of 10 hours depending on level of activity. The placement of the traps at individual sites was identical during each visit to reduce any bias in catching efficiency. As swarming behaviour can be influenced by changes in the weather (Fenton, 1969, Parsons et al., 2003b) all of the surveys were carried out on nights with no precipitation, although the level of cloud cover often varied within and between nights. As previous studies have found that moon phase does not have any detectable influence on swarming activity (Karlsson et al., 2002, Parsons et al., 2003b), the date of swarming capture was not set up to coincide with any specific lunar phase.

Once caught, bats were removed from the traps and placed in cloth 'bat bags' before being processed at an area away from the swarming site to avoid further disruption. The time of capture, the sex, age, species, weight, forearm length and reproductive condition was recorded for every bat caught. The age of the bat was determined as either adult or juvenile following the degree of ossification of the epiphyseal joints of the finger bones

(Dietz and von Helversen, 2004) and through the presence/absence of the dark spot on the lower lip (*M. bechsteinii* only) (Richardson, 1994). By late autumn this is not always possible so where there was some uncertainty to the bats age is was class as 'adult'. Reproductive condition of male bats was identified by checking the size of the testes and epididymis colouration (Racey, 1982, 1988). After biometric details were obtained each bat was then released at the processing point.

Because this study is focussing on the level of bat activity at the different sites and previous capture-mark-release studies have shown that there is a high turnover of bats from night to night (Parsons et al., 2003a, Rivers et al., 2006) with individuals only staying at the site for a few hours (Parsons et al., 2003b), none of the bats caught were ringed for identification purposes.

Data analysis

In order to compare the swarming behaviour of bats observed in this study to those previously carried out the data from all of the sites were combined, as has been done before with sites surveyed in a small area (Parsons et al., 2003a, Rivers et al., 2006). As the length of time spent catching bats varied between the sites at Acton and Winspit the results were standardised by dividing the number of bats caught by the number of hours the traps remained up after sunset (time of sunset taken from Meteorological Office weather data). To test for any difference in bat activity between the different sites, the mean number of bats caught per night per hour was calculated. When analysing the species composition and abundance between the sites the percentage of individual species caught per night at each site and percentage distribution of species caught between sites was calculated.

Results

A total of 838 bats of 8 species were caught over 64 capture occasions at the five study sites. The first swarming survey at Winspit was missed in September 2005 as permission had not been granted to access the site and no surveys were carried out at Edmunds quarry in 2007 due to the site being flooded.

Using the time of capture and amount of clay covering the bats as an indicator, a total of 42 vespertilionid bats caught (5%) were identified as using the quarries during the day. The majority of these catches were made at Burngate (60%) with other individuals caught at Winspit (28%) and Bowers (12%). *M. nattereri* was the most common species found using the quarries during the day (69%) although *M. daubentonii* (19%), *M. brandtii* (7%) and *M. mystacinus* (5%) were also caught. The sex ratio of these bats was highly male biased (Table 11: G-test, $P < 0.05$). Many *R. ferrumequinum* were caught leaving the quarries early in the night indicating that these bats are using the quarries during the day. This is supported by underground surveys, carried out previous to this study, that found *R. ferrumequinum* using the quarries throughout the year (Stebbings, 2003). These records were removed from the dataset in order allow for analysis of swarming activity of vespertilionid bats to be carried out at the different quarries.

Comparisons with previous swarming surveys

Species composition

The results of this study show a general concordance with previous swarming studies carried out in Europe and North America. Although species composition varied between sites, the majority of bats caught at the swarming sites were from the genus *Myotis* (86%) (Table 11). *M. nattereri* was the most dominant *Myotis* species recorded at all sites (56%) (Fig. 15) with the exception of Winspit where it was similar to the number of *M. mystacinus* caught (Table 11).

Timing of swarming

Swarming activity was observed from the beginning of September until the end of October, peaking between the middle of September to early October at all of the inland quarries at Acton although results indicate that swarming activity may have begun earlier at Winspit due to the high numbers of bats caught in early September.

Sex and age composition

The sex and age-ratio was found to be significantly male and adult biased in seven of the eight species tested (G-tests, $P < 0.05$) (Table 11). Only in *M. brandtii* were these found not to differ significantly from unity (G-test, $P > 0.05$), but this is most likely due to the small sample size ($n = 14$). *E. serotinus* was not tested as only two males were caught during the whole study. Although the age ratio of *R. ferrumequinum* was found to be male biased across all sites, significantly more juveniles were caught at Winspit compared to all of the other sites, with the ratio being closer to unity at Bowers (Table 12). Pooling the data from all of the vespertilionids found that all of the sites followed the same trend as seen across species and had an adult-biased age ratio.

Comparison of different sites

Comparison of bat activity between the Acton quarries and Winspit

Swarming activity varied greatly between sites. The greatest number of bats caught at any one site was at Burngate when 97 bats were processed on the 3rd October 2005 over 9 hours. The highest capture rate was at Winspit where 13 bats/trap/hour was recorded on the 22nd September 2007. Pooling the data over all survey periods, Winspit was found to have the highest capture rate and Edmund's the lowest (Fig. 16). To test for any differences in bat activity between the different sites the data collected from Edmunds quarry were removed due to low sample size. The number of bats/hour/night were then normalised using logarithmic transformations and then tested using one-way Analysis of Variance (ANOVA). There was a significant difference in the number of bats caught between the quarries tested (ANOVA, $F = 10.02$, d.f. $= 3$, $P < 0.001$) with Turkey's test revealing that the capture rates were not significantly different between Winspit and Burngate or Bowers and Brownsea, but there was a significant difference between the two groups (Fig. 16).

Due to the low number of bats caught at Edmunds quarry on each sampling occasion the data could not be normalised so the number of bats caught each night was pooled for 2005 and 2006 (years Edmunds quarry was surveyed) enabling a non-parametric test (G-test) to be used. As all of the quarries at Acton were surveyed for the same number of hours on each sampling occasion between 2005 – 2006, a direct comparison between sites was possible without having to adjust for the number of hours each trapping session lasted. The number of bats caught at Edmunds between 2005 – 2006 was significantly different from the number of bats caught at Brownsea quarry (the second lowest catch between 2005 – 2006) (G-test, d.f. = 1, $P < 0.001$). Because all of the other quarries had been tested at Acton no further analyses were carried out.

Species richness and abundance between quarries

Species richness was used instead of diversity indices (e.g. Simpson's Diversity Index) due to the low sample size of bats caught at some of the quarries. Ranking the quarries according to their species richness produced the same trend as was seen for capture rate, with Winspit having the greatest species richness and Edmunds the lowest:

Winspit (8) > Burngate (7) > Bowers (6) > Brownsea (4) > Edmunds (2)

Winspit made up more than 50% of the records for *E. serotinus*, *M. bechsteinii*, *M. brandtii*, *M. daubentonii* and *M. mystacinus*. It also contributed a large proportion of the number of *R. ferrumequinum* caught (42%) (Fig. 17). With the exception of Winspit, *M. nattereri* made up the majority of catches at all sites (50 – 83%) with Burngate quarry contributing half of the number of records over the three years (Fig. 18).

Where possible, testing for differences in species abundance between the different sites was carried out using log transformed data of the number of bats/hour/night using one-way ANOVA's. When this was not possible, non-parametric tests were used. Comparing the species abundance between Winspit and Burngate, the two sites with the greatest overall level of bat activity, found that there was no significant difference in the number of *R. ferrumequinum*, *M. bechsteinii*, *M. nattereri* and *P. auritus* visiting the two sites (Fig. 19). Significantly higher numbers of *M. brandtii* and *M. mystacinus* were caught at Winspit compared to all of the other quarries. Although Winspit had significantly higher overall capture rates than Bowers or Brownsea, there was no

significant difference in the number of *M. nattereri* caught between the three sites, or between Winspit and Bowers when comparing *P. auritus* abundance (Fig. 19).

Species abundance varied within and between the two quarry types at Acton. The two unrestored quarries (Burngate and Bowers) did not significantly differ in capture rates for any species except for *M. nattereri* where significantly higher numbers were recorded at Burngate. Due to the low species richness at the two restored quarries (Brownsea and Edmunds) and the low number of bats caught at these two sites, G-tests were carried out to compare for differences in abundance of *R. ferrumequinum*, *M. bechsteinii*, *M. nattereri* and *M. daubentonii*. Brownsea was found to have significantly higher *M. nattereri* and *M. daubentonii* abundance (G-test, $P < 0.05$).

Significantly higher numbers of *R. ferrumequinum*, *P. auritus* and *M. mystacinus* were caught at the unrestored quarries when compared to the restored quarries. There was no difference in the number of *M. bechsteinii* or *M. daubentonii* between the two unrestored quarries and Brownsea or between Bowers and Brownsea when comparing the number of *M. nattereri* caught.

Table 11. Total number of bats of each species caught during catches at the five different sites between 2005 – 2007 and % males for each species with pooled study sites. * denotes a sex ratio significantly different from unity ($P < 0.05$) after Bonferroni correction for multiple tests. [NS] denotes a sex ratio not significantly different from unity ($P > 0.05$). Species abbreviations are given below.

Site	Number of catches	R. f	E. s	M. b	M. br	M. d	M. m	M. n	P. a	Total caught
Burngate	14	32	0	19	1	8	3	239	6	308
Edmunds	9	0	0	1	0	0	0	5	0	6
Bowers	14	18	0	7	0	5	2	42	9	83
Brownsea	14	1	0	9	0	6	0	56	0	72
Winspit	13	37	2	43	13	31	92	102	7	327
Total	64	88	2	79	14	50	97	444	22	796
% males		69.4*	100.0[a]	76.6*	64.7[NS]	80.0*	75.6*	69.7*	75.0*	72.3*[b]
% adult			100.0[a]	76.6*	50.0[NS]	83.0*	67.0*	86.5*	80.0*	81.4*[b]

R. f *Rhinolophus ferrumequinum*, E. s *Eptesicus serotinus*, M. b *Myotis bechsteinii*, M. br *Myotis brandtii*, M. d *Myotis daubentonii*, M. m *Myotis mystacinus*, M. n *Myotis nattereri*, P. a *Plecotus auritus*.

[a] G-test was not carried out due to low sample size

[b] Test carried out excluding *R. ferrumequinum* samples, n = 708

Table 12. % number of adults for *R. ferrumequinum* and all other species caught at the different underground sites. * denotes a sex ratio significantly different from unity ($P < 0.05$) after Bonferroni correction for multiple tests. [NS] denotes a sex ratio not significantly different from unity ($P > 0.05$).

Site	% adults			
	R. ferrumequinum		Vespertilionids	
Burngate	70.3*	(32)	83.8*	(276)
Edmunds	-	(0)	100.0*	(6)
Bowers	55.5[NS]	(18)	77.6*	(65)
Brownsea	100.0[a]	(1)	96.8*	(71)
Winspit	20.5*	(37)	78.0*	(290)

Vespertilionids contains: *Eptesicus serotinus*, *Myotis bechsteinii*, *Myotis brandtii*, *Myotis daubentonii*, *Myotis mystacinus*, *Myotis nattereri*, *Plecotus auritus*.

[a] G-test was not carried out due to low sample size

Figure 15. Species composition of all bats caught for all sites between 2005 – 2007. n = 796.

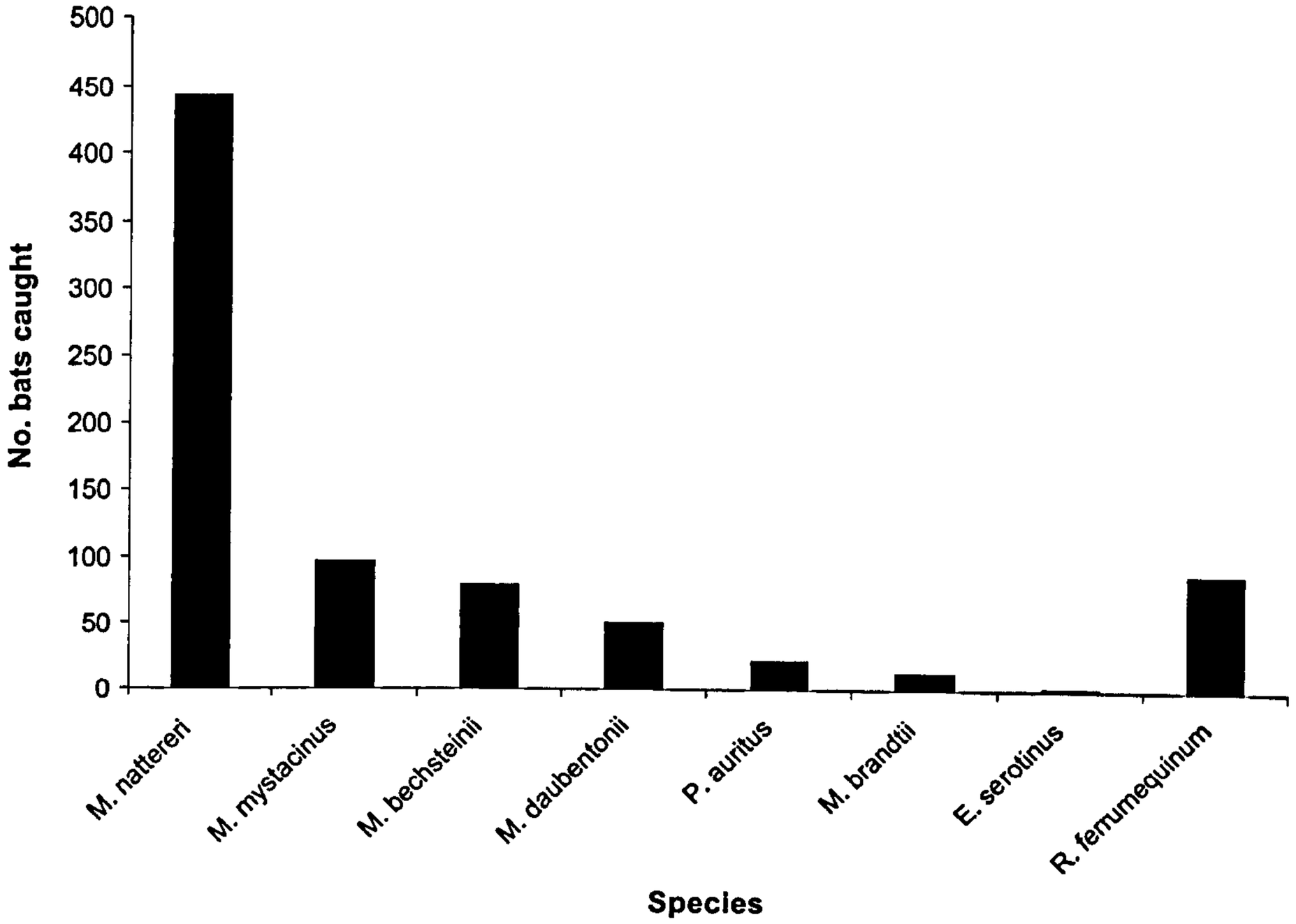


Figure 16. Mean number of bats caught per hour per night during the course of the study at the five different sites (± SE). Quarry type indicated by colour: Restored = black, Unrestored = grey, Sea = hatched. Number of swarming catches shown in brackets. Quarries that support levels of activity that are not significantly different from one another are indicated by the same letter.

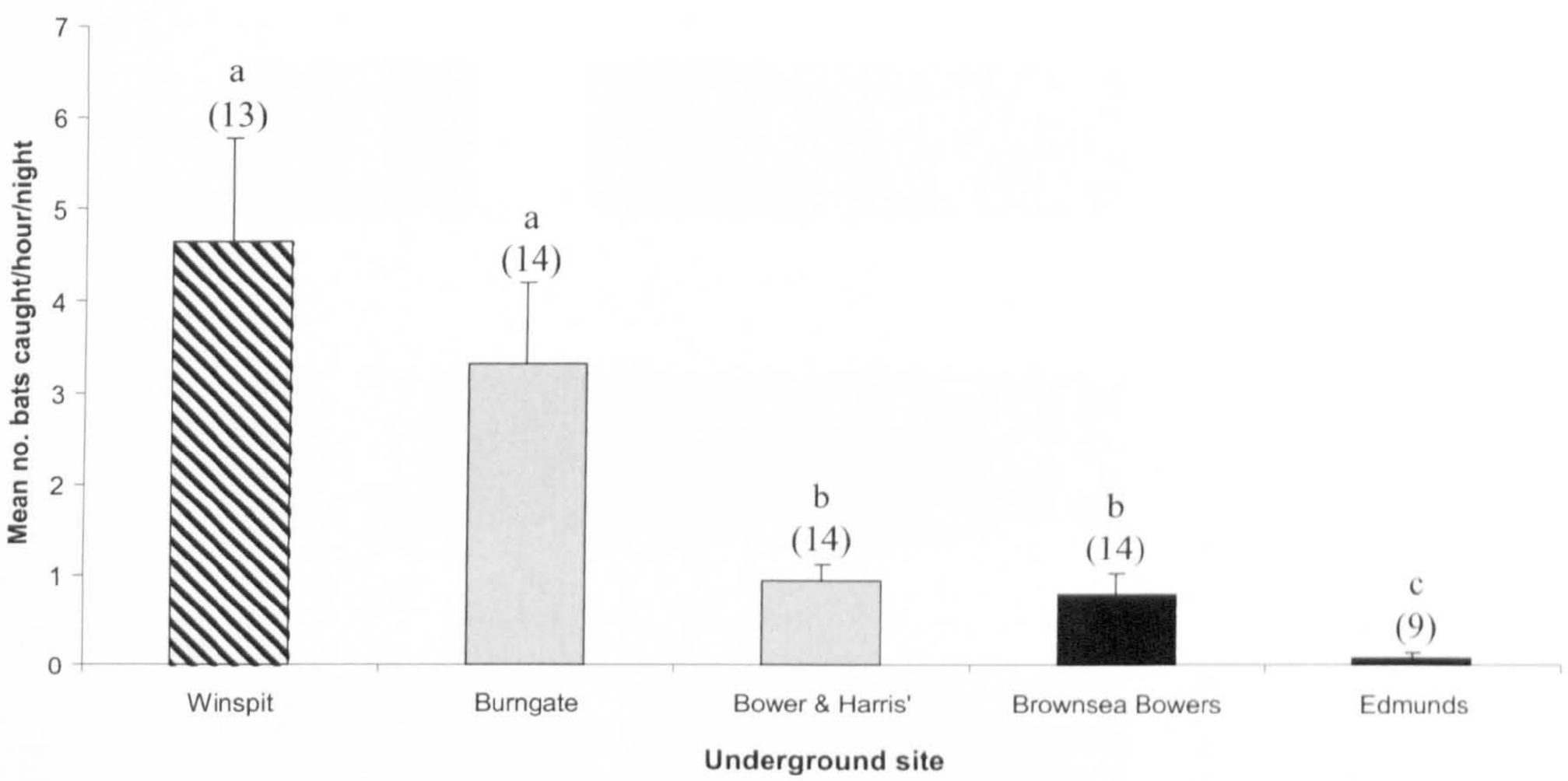
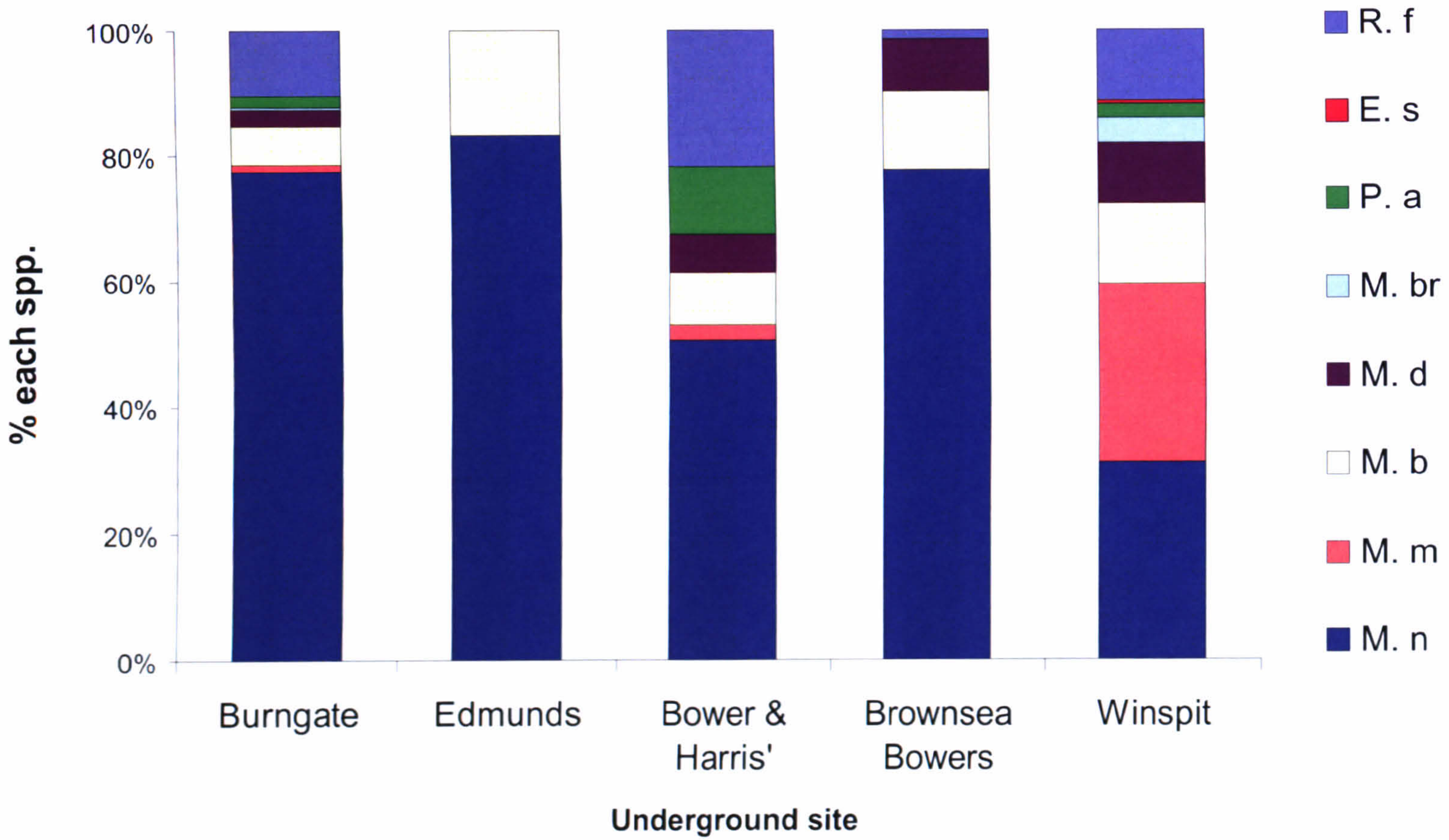
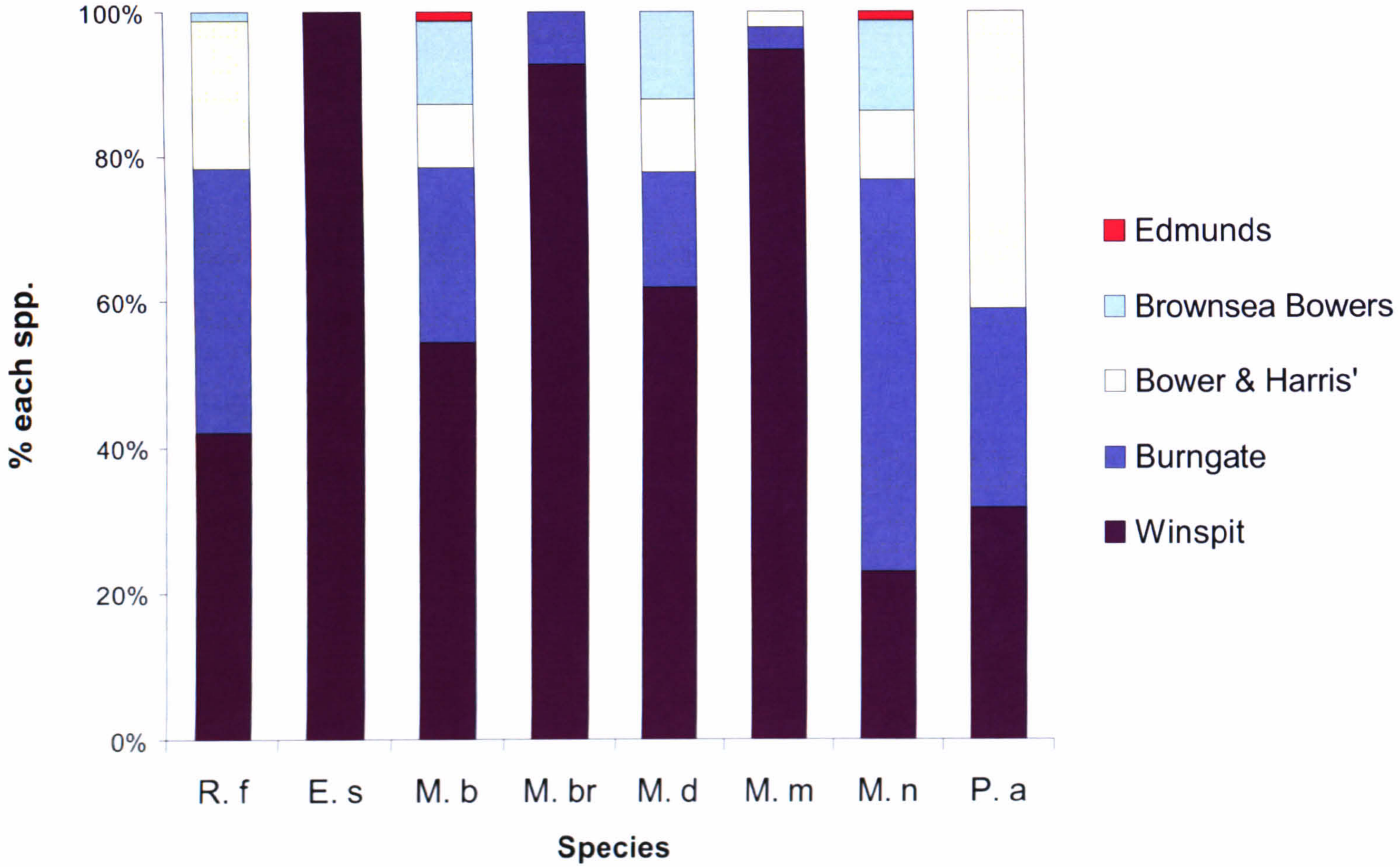


Figure 17. Percent species composition at each site between 2005 – 2007.



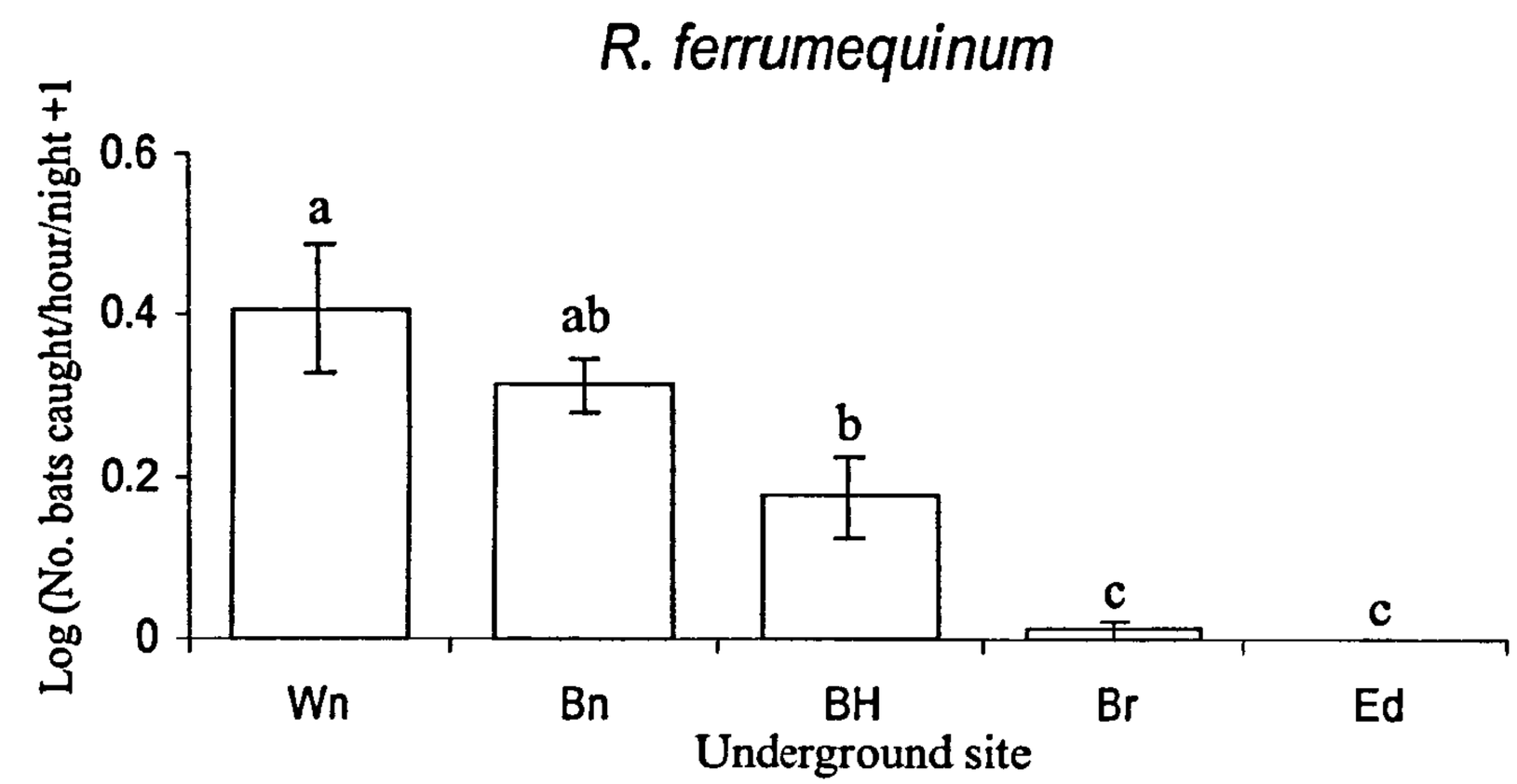
R. f *Rhinolophus ferrumequinum*, E. s *Eptesicus serotinus*, M. b *Myotis bechsteinii*, M. br *Myotis brandtii*, M. d *Myotis daubentonii*, M. m *Myotis mystacinus*, M. n *Myotis nattereri*, P. a *Plecotus auritus*.

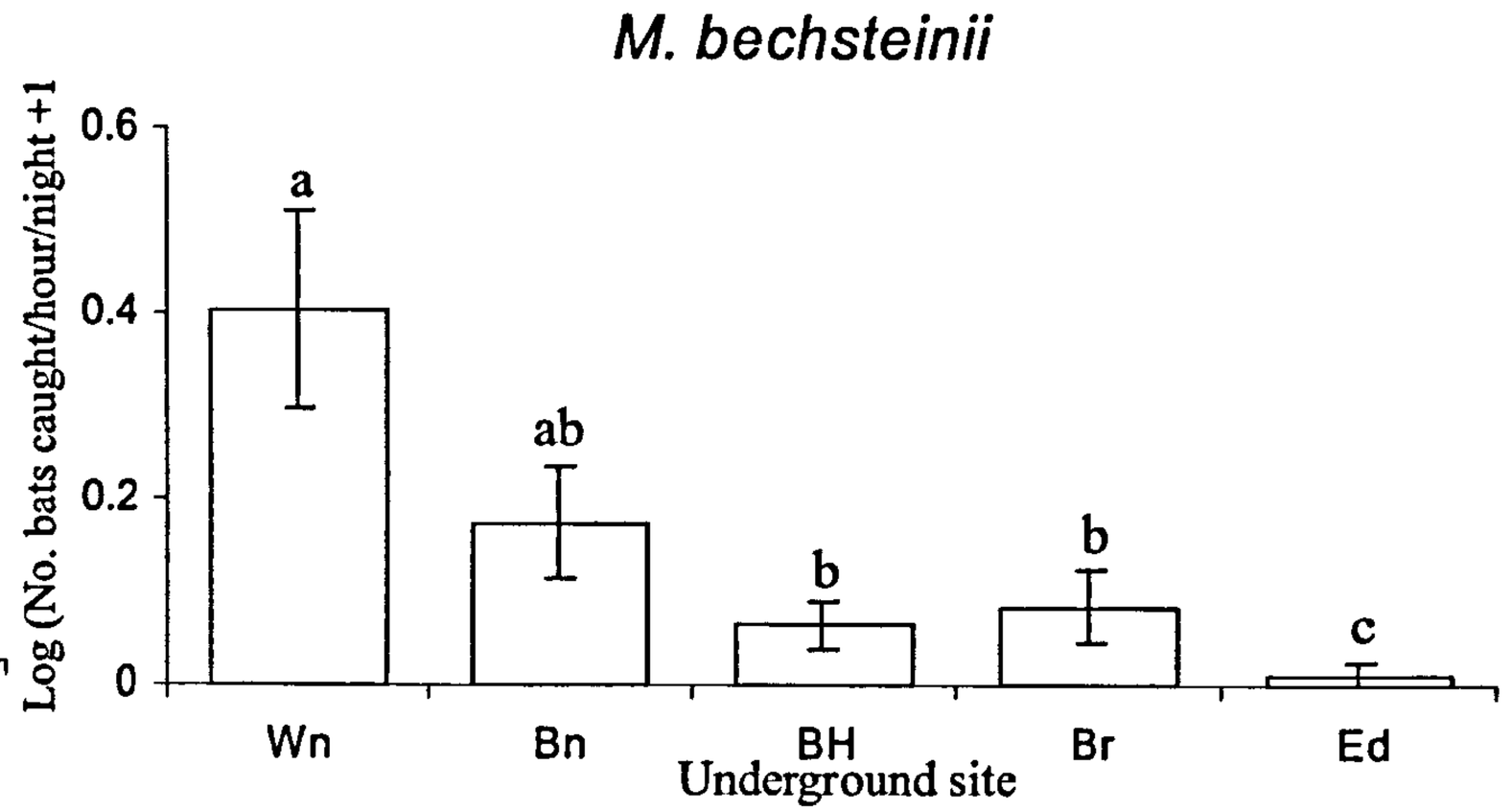
Figure 18. Percent distribution of species caught at the different sites between 2005 – 2007

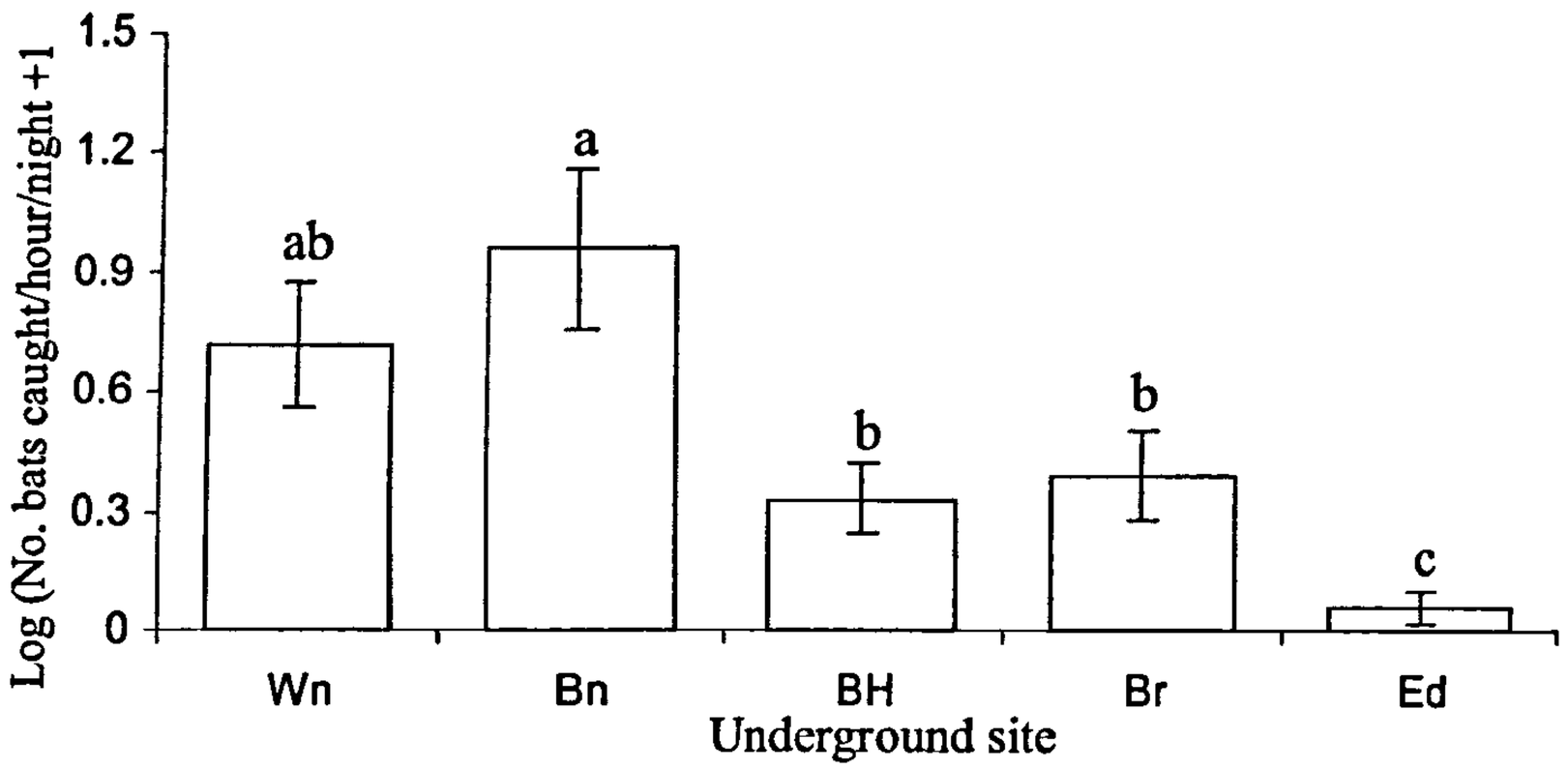


R. f *Rhinolophus ferrumequinum*, E. s *Eptesicus serotinus*, M. b *Myotis bechsteinii*, M. br *Myotis brandtii*, M. d *Myotis daubentonii*, M. m *Myotis mystacinus*, M. n *Myotis nattereri*, P. a *Plecotus auritus*.

Figure 19. Adjusted mean log transformed counts of total bat catches made at each sites (± SE). Underground sites that support levels of activity that are not significantly different from one another are indicated by the same letter.







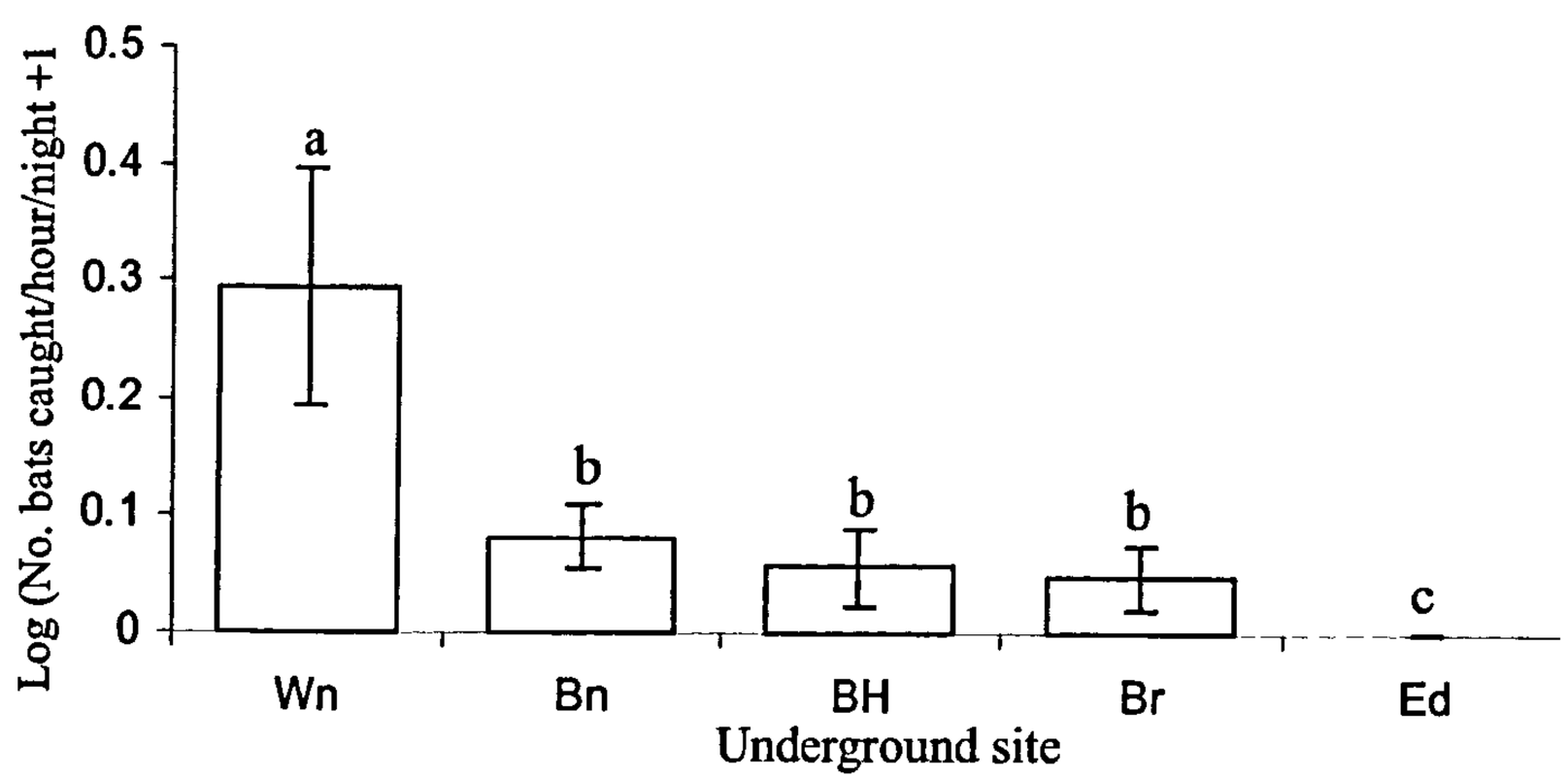
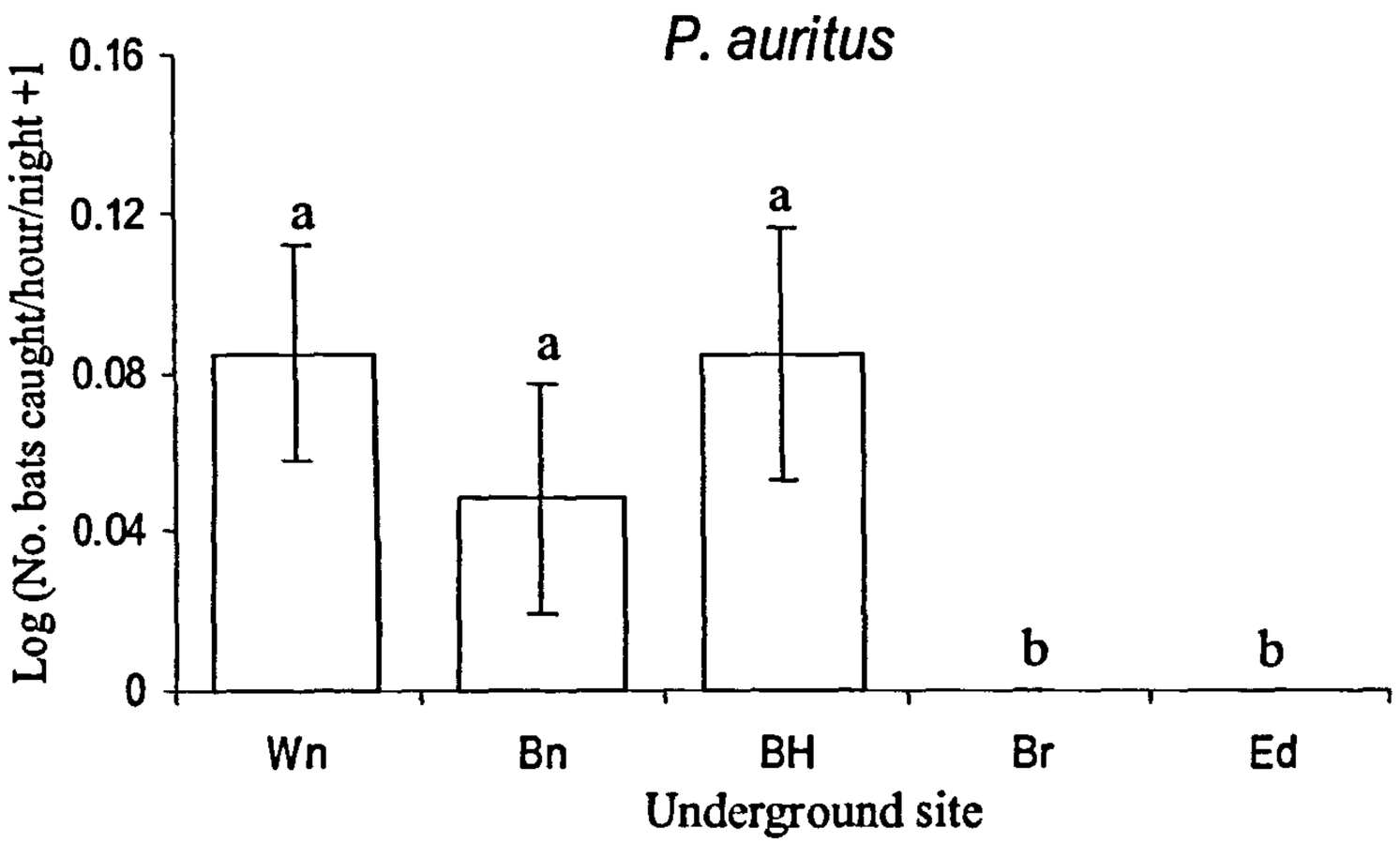
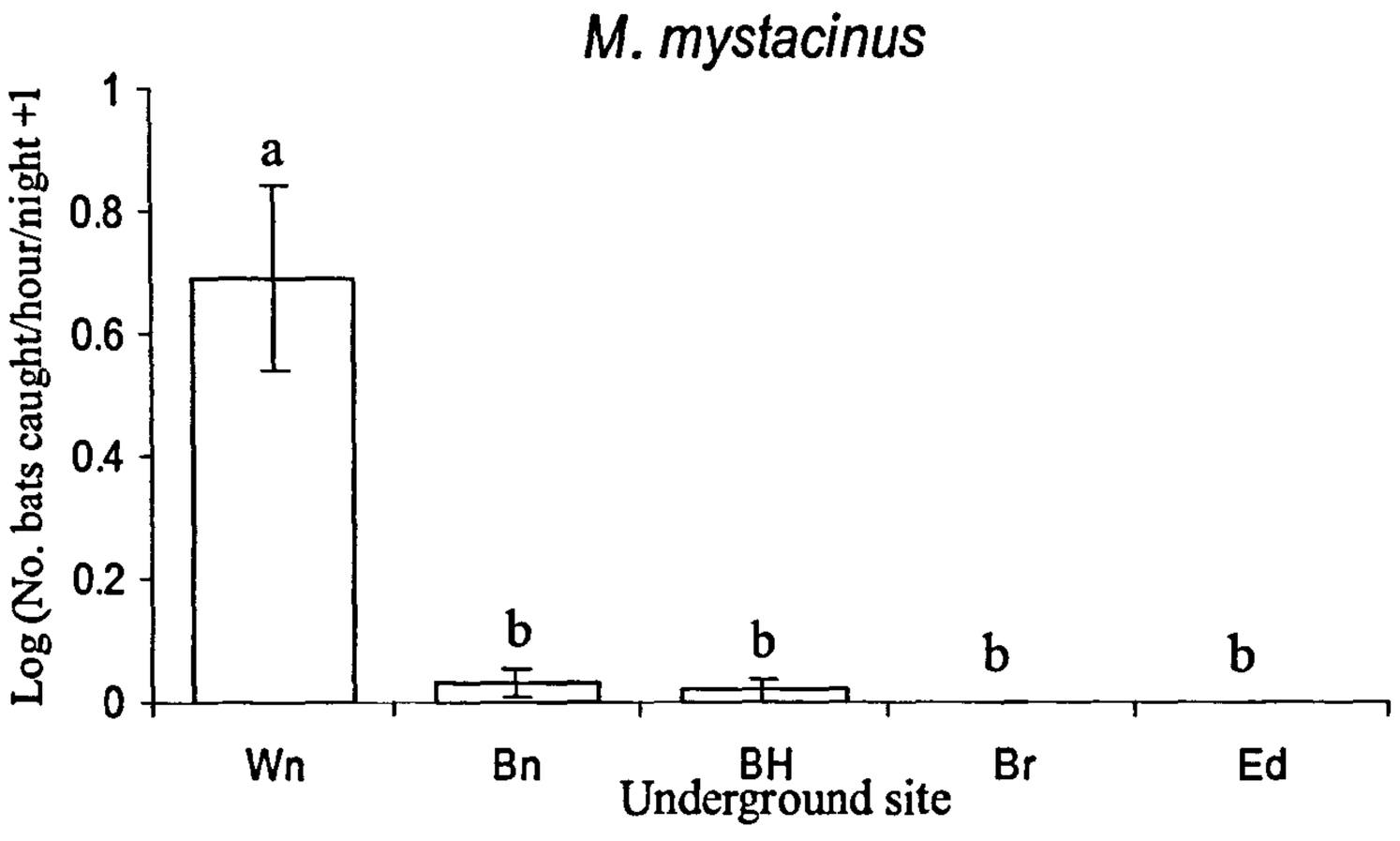
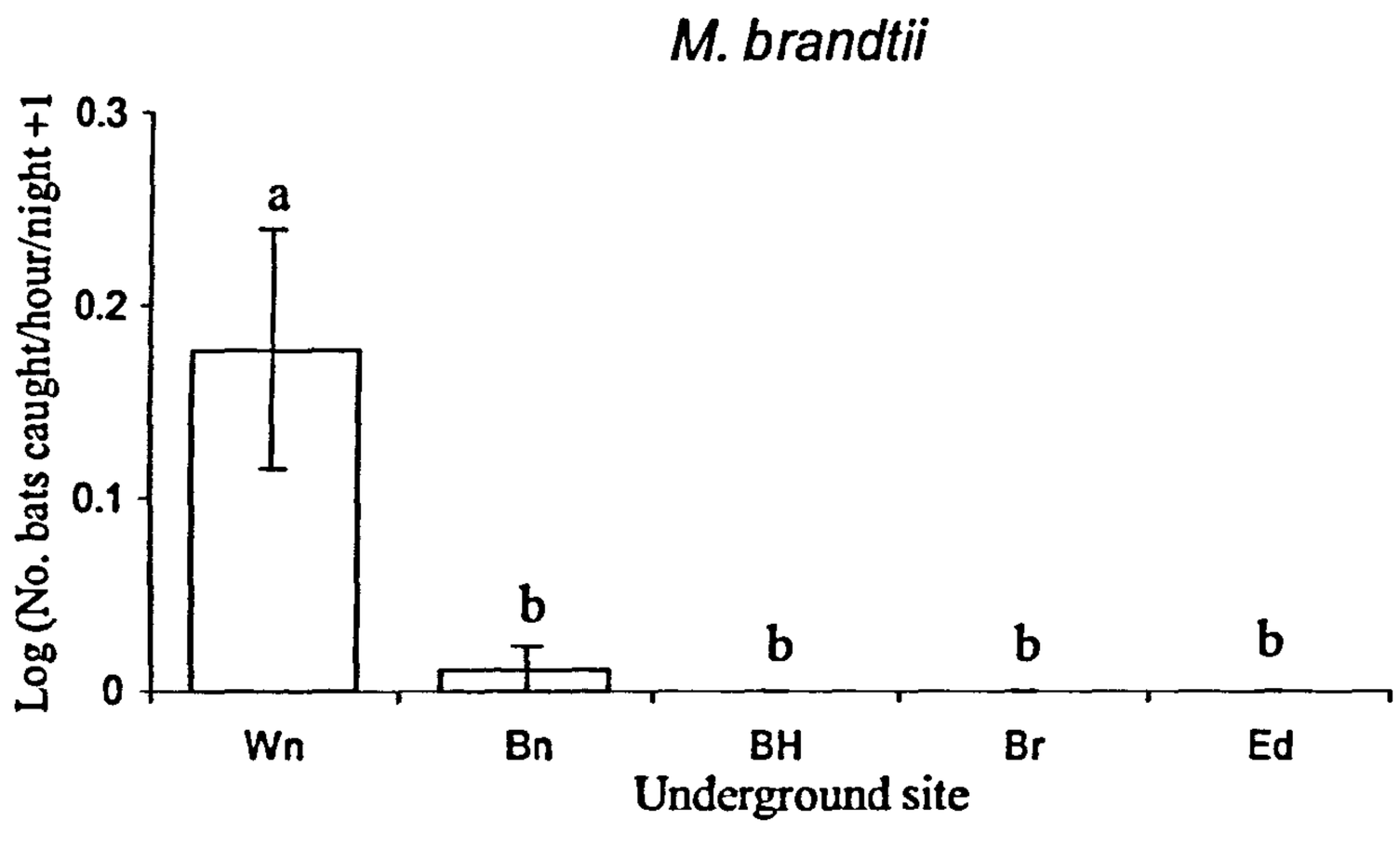
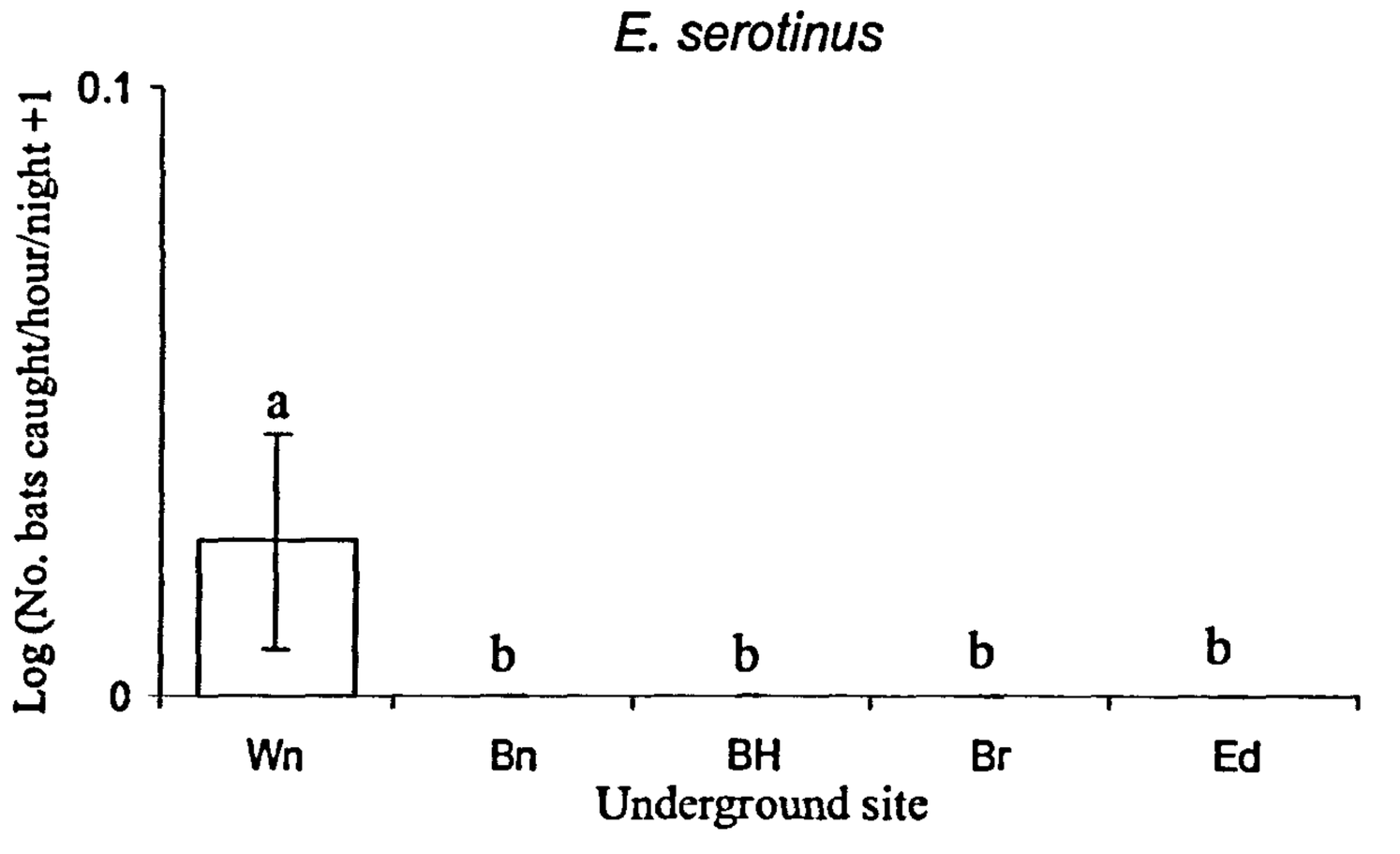


Figure 19. Cont.









Discussion

Swarming activity in Purbeck

The swarming activity observed across all of the sites in Purbeck was similar in nature to that seen in studies carried out in Europe and the United States (e.g. Fenton, 1969, Bauerova and Zima, 1988, Parsons et al., 2003a, Rivers et al., 2006). Swarming was most prevalent among bats in the genus *Myotis*, with *M. nattereri* making up over half of the total number of bats caught over the course of the study. Populations of bats at swarming sites were heavily male-biased in all species apart from *M. brandtii*, but this was most probably due to the low sample size for this species.

Compared to an earlier swarming study carried out in the south of England (Parsons et al., 2003a), the relative number of *M. daubentonii* caught in this study was extremely low. Although species composition will depend on geographical location (Rivers et al., 2006), one would have expected a greater number of *M. daubentonii* visiting the quarries. Similarly, *M. mystacinus* being the second most abundant species caught in Purbeck, was also unexpected when compared to the work by Parsons et al. (2003a), especially considering its abundance relative to other *Myotis* species (Harris et al., 1995). These results would suggest that either *M. mystacinus* is locally abundant, especially with regard to *M. daubentonii*, or that it was actively selecting to swarm at Winspit. The importance of surveying a large number of swarming sites is shown here because if this study had only concentrated on the quarries at Acton it would have led to similar conclusions to Parsons et al. (2003a) as *M. daubentonii* records at the Acton quarries exceed *M. mystacinus* records by a ratio of 4:1.

Bat activity was greatest at all of the Acton quarries between the middle of September to early October but occurred earlier at Winspit. Swarming studies were not carried out early enough at Winspit to confirm when the peak in activity occurred but the results show that bat abundance was greatest in the first few weeks of September compared to later on in the month. The difference in activity levels could be due to the difference in species composition between Acton and Winspit. Parsons et al. (2003a) found that there was temporal variation in the abundance of different species with *M. mystacinus* activity peaking before that of *M. daubentonii* and *M. nattereri*. Considering the relatively high

abundance of *M. mystacinus* at Winspit compared to the Acton quarries, this may explain the earlier peak in activity.

The high male bias in the number of bats caught, many of which were sexually mature, supports the theory that mating is an important part of swarming behaviour (Fenton, 1969, Thomas and Fenton, 1979, Kerth et al., 2003, Parsons et al., 2003a, Veith et al., 2004, Rivers et al., 2005). If this is the only reason why the bats are visiting the quarries is still a matter of debate and not one of the main aims of this study. As with previous studies, the presence of juvenile, sexually immature bats of both sexes at the swarming sites suggests that some form of information transfer is occurring. This could suggest that swarming may have more than one function as previously suggested by Bradbury et al. (1986) or it may be that the juveniles are simply following the adults and learning the location of mating sites that they can use upon reaching sexual maturity.

The low number of vespertilionids roosting in the quarries during the day suggests that they are not being used as migration stop-over points but does not rule out the possibility that the sites are acting as focal points for migrating bats (Whitaker, 1998). As the majority of the bats caught swarming will roost in trees or man made structures such as houses or underneath bridges during the summer months, quarries may act as a gathering point for populations that may otherwise be isolated for most of the year.

Hibernation counts have found relatively few *Myotis* or *Plecotus* species using the quarries (Stebbings, 2003). This suggests that assessing suitable hibernacula and information transfer of suitable hibernaculum between adults and juveniles is not the primary reason behind swarming (Davis and Hitchcock, 1965, Fenton, 1969, Cope and Humphrey, 1977). It is not uncommon to record very few bats in hibernacula as crevice dwelling species such as *M. nattereri* are difficult to find at underground sites (Cerveny and Horácek, 1981). A number of studies have demonstrated this by recording greater numbers of bats emerging from underground sites during the spring than were counted during winter counts (Gilbert and Stebbings, 1958, Rehák et al., 1994, Parsons et al., 2003a, Rivers et al., 2006). Although more bats are likely to be hibernating in the quarries than are recorded, the clay lined walls of the inland quarries at Acton will greatly reduce the number of crevices available and may reduce their suitability as hibernacula.

The capture of a *M. nattereri* in 2005 that had been marked with a ring and caught over successive summers in Wareham forest (M. Warn pers. comm.) shows that the quarries have a catchment area of at least 16 km (straight line distance from Acton to Wareham forest). Previous studies in the UK have shown that swarming sites can have large catchment areas. By radio-tracking *M. nattereri* and *M. daubentonii* from swarming sites back to their roosts, Parsons and Jones (2003) found that the mean distance travelled by these two species was 11.9 km (max. 36.2 km) and 18.7 km (max. 35.1 km) respectively while Rivers et al. (2006) found that a *M. nattereri* travelled around 60 km.

As none of the bats in this study were fitted with rings or radio-tags, it is impossible to say over what distance many of the bats travelled to get to these sites. Although there are very few traditional inland quarries outside of Purbeck other sites such as disused railways tunnels or sea caves can be used instead (Parsons et al., 2003a).

Comparison of swarming communities between different sites

The main disadvantage of surveying a large number of different quarries on the same night was the impracticality of ringing all of the bats caught. Consequently, this study is not able to support or reject the findings of previous studies that suggest bats are faithful to an individual swarming site (Fenton, 1969, Parsons and Jones, 2003). Rivers at al. (2006) suggest that bats may visit more than one site in an evening if the swarming sites are within a short distance of one another (< 2 km to nearest neighbour in this study) and that these sites can be considered as a single swarming 'area'. The variation in bat activity at Acton shows that although we cannot identify whether bats visited more than one site in a single evening, these results illustrate that a greater number visited just one quarry (Burngate) than any of the others, especially with regard to *M. nattereri*. It is important that if a number of underground sites within a small area are to be grouped together as a single swarming 'area', each site is assessed on an individual basis before a full understanding of the population dynamics of the area is known.

Comparison of restored and unrestored quarries

Comparing the level of bat activity at the different quarries at a species specific level shows that there were some differences between the restored and unrestored quarries. *P. auritus* was not caught at either of the two restored quarries but was recorded at the two unrestored sites. A similar trend is seen with *R. ferrumequinum* as only one individual was caught at Brownsea with none at Edmunds. As *R. ferrumequinum* is not a swarming species and is found using the quarries during day, it is not surprising that no bats individuals were caught at Edmunds due to its tendency to flood during wet weather. Only one *R. ferrumequinum* caught at Brownsea over the course of the study was unexpected as historically individuals have been using this site in similar numbers to Burngate and Bowers (Stebbings, 2003). Although the capture of one individual shows that *R. ferrumequinum* is still able to use the site, it is possible that reducing the size of the entrance during restoration has made this site less suitable for this species. This may be directly as a result of bats being impeded in freely entering/exiting the site (White and Seginak, 1987, Ludlow and Gore, 2000, Pugh and Altringham, 2005, Spanjer and Fenton, 2005) or indirectly though changes in the thermal characteristics due to the limited air flow (Richter et al., 1993).

Comparison of inland quarries at Acton with Winspit

Although Winspit was not surveyed on the same night as the Acton quarries, both sites were surveyed on nights with similar weather conditions. By calculating the mean number of bats caught per night per hour over the whole three year of this study, the effect of nightly variation in bat activity should be reduced.

Winspit had the greatest species richness and had a significantly higher bat capture rate to any of the quarries at Acton except for Burngate ($P < 0.05$). A possible reason for this could be that higher species diversity has previously been reported at more isolated swarming sites (Johnson et al., 2006). However, although Winspit was the only coastal mine surveyed in this study, it is one of many similar sites along the coast, some of which are within ca. 200 m. Records showing that coastal mines have consistently had higher *R. ferrumequinum* abundance (Stebbings, 2003) may indicate that these sites are more suitable for this species.

Implications for conservation

Due to the difficulties of surveying crevice dwelling species, surveying bats at swarming sites can provide more information than if winter hibernation checks are made on their own (Parsons et al., 2003a, Rivers et al., 2006). Although catching bats at swarming sites will cause high levels of disturbance, and should be limited in the number of times they are carried out at any one site, it is the only method to ascertain true species composition, sex biases and sexual maturity. Catching bats at this time of the year may be less invasive than handling them during hibernation as the arousal of bats may lead to the expenditure of valuable energy reserves (Thomas, 1995). Other methods such as automatic logging and infrared observations are useful when looking at activity levels but are limited in their inability to accurately distinguish between different species, especially within the *Myotis* genus.

This study has shown the importance of protecting as many underground sites as possible as some may be used more by one species than another. *M. nattereri*, a species whose UK population of is considered to be of international importance (Stebbings, 1988, Hutson, 1993, Macdonald and Tattersall, 2001), clearly selected to swarm at Burngate and Winspit so the loss of either of these sites could be detrimental to the species. Similarly, the loss of Winspit could have a big impact on *M. mystacinus*, a species that is considered 'vulnerable' at a national level (Hutson, 1993, Macdonald and Tattersall, 2001). Until now there have been a limited number of *M. mystacinus* records in Purbeck, this study shows that this species may be more numerous than previously thought.

Altering a potential swarming site must only be made after extensive monitoring of the site has been carried out. Although the reasons for swarming, and the factors influencing site selection are still not fully understood, as bats will often return to the same swarming site (Rivers et al., 2006), altering a site may have severe implications to the bat communities using them. This may by through disturbance, thermal variability change or loss of cover. If a site does need to be restored no more than one site should be altered at any one time to minimise the amount of disturbance caused. Only by improving our understanding of the characteristics that swarming communities select can better-informed management decisions be made in the future.

Molecular phylogenetics and phylogeography of the greater horseshoe bat *Rhinolophus ferrumequinum*

Summary

The impact of glaciations on the distribution, population structure and genetic diversity of populations is well established. In Europe, the main refugia identified are Italy, Iberia and the Balkans. However, most studies do not sample beyond Europe even when the species range is much wider, potentially leading to an ascertainment bias. Furthermore, phylogeographic studies are typically based on haplotype data, occasionally on nuclear markers such as microsatellites, but rarely combine multiple markers. This is unfortunate because the use of markers with contrasting modes of inheritance and rates of evolution might provide a more accurate and comprehensive understanding of a species' history. The following chapter presents a detailed study of the phylogeography of the greater horseshoe bat *Rhinolophus ferrumequinum* by using mtDNA collected from forty-five localities from across its Palaearctic range, including the Isle of Purbeck, Dorset (UK), to infer population history. These data are then compared to microsatellite data already available for this species. It shows that the phylogeographic signals obtained from the two markers are different and that only by combining the datasets is it possible to reconstruct the species' history through multiple glacial periods, and assess the relative importance of European versus Asian refugia. In Europe, the results suggest two colonisation events, one before the Last Glacial Maximum (LGM) and another after it. Analysed separately, neither marker was able to show both events, or what influence Asia Minor has had in influencing population genetic structure of the individuals in this region. If interpreted using mtDNA data alone, it would not have been possible to identify any specific European glacial refugia or colonisation routes after the LGM. In east Asia, due to high levels of divergence between populations, mtDNA data proved the most useful due to the effects of homoplasy on the microsatellite data. High levels of diversity indicate there was at least one major refugium in east Asia and that there was an early colonisation of Japan well before the LGM.

Introduction

Biogeography is an integrated discipline that combines ecological and phylogenetic data to provide information on the origin, evolutionary history and current distributions of species (Wiens and Donoghue, 2004). Molecular techniques are now being increasingly used to help explain species lineages and global patterns of biodiversity by drawing inter- and intra-specific phylogenies of the taxa being studied. When studying intra-specific phylogenies of small, fragmented populations this can be especially important because barriers to gene flow can be identified and conservation implementations proposed to limit any further loss of genetic variation (Rossiter et al., 2000). Recent studies have demonstrated the effectiveness of molecular techniques in conservation management by identifying isolated populations of *R. ferrumequinum* (Rossiter et al., 2000, 2001), barbastelle (*Barbastella barbastellus*) (Juste et al., 2003), Bechstein's bat (*Myotis bechsteinii*) (Mayer and Kerth, 2005, Kerth et al., 2008), noctule (*Nyctalus noctula*) (Petit, 1998, Petit and Mayer, 1999, 2000), and the greater mouse-eared bat (*Myotis myotis*) (Ruedi and Castella, 2003, Ruedi et al., 2008).

Global distribution of the *R. ferrumequinum*

R. ferrumequinum is the most widely distributed species of the *Rhinolophus* genus in the Palaearctic (Servent et al., 2003). It is found in south western regions of England and Wales, in north western France, across southern Europe, the Middle East and northern Africa, central Asia and Japan (Ransome, 1980). The evolutionary history of bats, including *R. ferrumequinum* is largely unknown due to the limited fossil record resulting in conflicting, incomplete and poorly supported phylogenies (Cooper et al., 1998, Teeling et al., 2005). Phylogenetic studies of the *Rhinolophus* genus (Servent et al., 2003) has shown that the most likely origin of *R. ferrumequinum* is from Africa some five million years ago. This probably occurred when an 'African clade' of the *Rhinolophus* genus was isolated from the rest of Europe and Asia due to climatic changes making impassable land barriers (Servent et al., 2003). When these land barriers eventually became easier to cross, *R. ferrumequinum* was just one of the species to have diverged from this clade of *Rhinolophus* bats (Servent et al., 2003). Since divergence occurred, the distribution of *R. ferrumequinum* would have been restricted

by habitat availability due to global climatic fluctuations altering the landscape (Roy et al., 1996, Hewitt, 2000, 2004a). Climatic variation was most pronounced during the Pleistocene epoch (1.8 my – 10,000 yrs BP) as the Earth experienced repeated glacial cycles over this period (Adams and Faure, 1997).

Impact of Ice Ages

The impact of glaciations on the distribution, genetic structure and diversity of populations is well established (Taberlet et al., 1998, Hewitt, 1999, 2000, 2004a). During glacial maxima many temperate regions would have been covered by ice or permafrost (Adams and Faure, 1997) forcing populations to contract into southern refugia until climatic conditions became favourable enough to allow northerly range shifts and re-colonisation events. In Europe, the main refugia identified are found in Italy, Iberia and the Balkans (Taberlet et al., 1998, Hewitt, 1999). Re-colonisation events from these refugia after the Last Glacial Maximum (LGM) (23-18 Ka) can be categorised by three broad colonisation events, named after the bear (*Ursus arctos*), hedgehog (*Erinaceus europaeus & concolor*) and grasshopper (*Chorthippus parallelus*) paradigms (Hewitt, 1999). The contraction and expansion of a species' range will have an impact on a population's genetic structure as repeated serial dilution of the gene pool, repeated founder effects, population bottlenecks and long distance migration will all lead to the loss of genetic variability (Nei et al., 1975, Ibrahim et al., 1996, Hewitt, 2000). Consequently genetic variability of a re-colonising population is believed to decrease with the increasing distance away from its source (Nei et al., 1975), and areas with relatively high genetic variability are commonly thought to represent glacial refugia (Avise, 2000).

Previous studies

The majority of phylogeographic studies have been based on a single type of marker, usually a mitochondrial gene (Moore, 1995, Avise, 2000). Because different markers each have their own unique genealogy, which might deviate from the species' history (Hewitt, 2004a), this approach can introduce problems. In particular, phenomena such as homoplasy, independent evolution, incomplete lineage sorting, biases caused by different modes of inheritance, effective population sizes (e.g. Chesser and Baker, 1996)

and sex-biased dispersal (Clutton-Brock, 1989, Colbert et al., 2001) can all lead to erroneous results and conclusions. One way to minimise the impact of such problems is to combine markers with different modes of inheritance and different rates of evolution (Hewitt, 2004a). The relative roles that historical and contemporary events have had on a species population structure can then be analysed with more confidence (Howes et al., 2006) as any potential ambiguity can be assessed and hopefully resolved according to the genes being used.

The choice of gene used in phylogeographic studies also has important implications for the timescale over which events can realistically be inferred. Hypervariable markers such as microsatellites, while useful for detecting contemporary gene flow, are widely considered less suitable for phylogeographical inference due to their tendency towards homoplasious mutations (Hewitt, 2004b). This is also a problem associated with rapidly evolving parts of the mitochondrion such as the control region, for which homoplasy can confound genetic distance estimation and phylogenetic inference over timescales beyond tens of thousands of years (Avise, 2000, Lloyd, 2003). Conversely, more slowly mutating markers (i.e. ND1 Lloyd, 2003) might be better for characterising ancient events, yet provide unsatisfactory resolution of patterns of intraspecific and phenotypic variation, which are necessary for revealing recent processes (Rokas et al., 2003). One way to address these relative shortcomings is to apply markers with contrasting mutation rates and modes of inheritance to the same dataset. For example, by using both mitochondrial DNA (mtDNA) and microsatellite data, a number of studies have now been able to gain further insight into how pre- and post glacial dynamics have influenced current population structure and genetic diversity in their chosen species (Seddon et al., 2001, Rokas et al., 2003, Heckel et al., 2005, Howes et al., 2006, Jadwiszczak et al., 2006).

Another common limitation to phylogeographical studies is one of restricted sampling of a species across its natural range. This can be seen in Europe where, even though many species range outside of Europe, sampling is often limited to European states. The reason for this is that the main emphasis of research continues to focus on the latitudinal movement of European taxa from southern refugia (Rokas et al., 2003) even though Asia Minor has been identified as a centre of diversity for many taxa (see Rokas et al.,

2003, Durka et al., 2005) and as a possible source of many European lineages including the hedgehog (Butler, 1988), gallwasp (*Andricus quercustozae*) (Rokas et al., 2003) and the bent-winged bat (*M. schreibersii*) (Bilgin et al., 2006). By sampling beyond Europe and considering wider longitudinal patterns in genetic diversity (Rokas et al., 2003) it might be possible to gain a more comprehensive population history for an individual species and enhance conservation management decisions in the future.

Rossiter et al. (2007) examined population history in *R. ferrumequinum* across its Palaearctic range using microsatellite markers. This study identified at least two refugial populations in Europe, expanding from southwest (Iberia and/or Italy) and southeast Europe (Balkans/Greece). Further subdivisions between Europe and Asia Minor, and the Middle East and Russia, highlighted other likely refugia during the LGM. Despite a decline in allelic diversity from the Middle East to the United Kingdom, suggesting a northwest expansion with climate warming, there was no evidence of post-LGM gene flow between Asia Minor and Europe. Therefore European populations of *R. ferrumequinum* appear to have experienced two episodes of expansion. In Asia, high allelic diversity in populations from northeast China and Japan raise the possibility of east Asian refugia, though discontinuities in allele frequencies within China might indicate that more than one species is present. Although these results provide important insights into the history of this species, they also highlight a need for supporting sequence data, so that population splits and expansion events can be dated.

The aim of this study is to address the limitations associated with phylogenetic analyses by comparing how genetic markers with different rates of evolution across a species' entire range can lead to contrasting demographic histories to be inferred. This is important in terms of conservation biology because incomplete phylogenies could mean that populations of high genetic diversity and historical importance are missed and left out of any management plans.

The objectives of this study are:

1. Determine the genetic variability of *R. ferrumequinum* populations from different geographical regions, including the Isle of Purbeck, Dorset, by sequencing the mtDNA ND2 gene.
2. Infer phylogeographic history of *R. ferrumequinum* using ND2 sequence variation.
3. Compare ND2 results to an extensive dataset from which phylogeographic history has already been inferred using microsatellites (Rossiter et al., 2007).
4. Assess the limitations of using single genetic markers to infer population demographic histories.

Methods

Sample collection

Tissue samples were collected or obtained from forty-five localities across the species range (Table 13, Fig. 20). The number of samples analysed varied due to sample size and sequencing success. Samples comprised either 3 mm wing membrane biopsies obtained (Worthington and Barratt, 1996) (Stiefel Laboratories, Wooburn Green, UK) or liver or muscle samples from museum samples. All tissue was stored at -20°C in 90% (v/v) ethanol.

DNA isolation and amplification

Genomic DNA was isolated from tissue by either using Qiagen DNeasy® Kits (Qiagen, Crawley, UK) or Promega Wizard® Purification Kits (Promega, Southampton, UK) according to the manufacturer's guidelines. The mitochondrial gene ND2 was amplified by polymerase chain reaction (PCR) using the primers L5074.M (5'-CTGATAAAAGARTTACTTTGATAGAG-3') (M. Sorenson, pers. com.) and H6305 (5'-GGCTTTGAAGGCYCTTGGTC-3') (Sorenson et al., 1999). This gene was selected because, in terms of amino acid sequence, ND2 is the third most variable gene in the mitochondria after ATPase 8, which is relatively short (~165 – 168 bp), and ND6 which is difficult to amplify due to its unusual base pair composition close to the control region (Sorenson, 2003). PCR's were performed in a 15 µl volume, containing approximately 3 – 30 ng of genomic DNA, 0.67 µM of each primer, 0.33 µM of each dNTP, 1.33 µM $MgCl_2$ and 1.0 µM of *Taq* polymerase (Bioline) in the manufacturers buffer. Reactions were performed on a DNA Engine Tetrad® thermal cycler (MJ Research, Waltham, USA) under the following conditions: 95°C for 15 min; 35 cycles of 95°C for 30 s, 60°C for 30 s, 72°C for 30 s; 72°C for 10 min. DNA sequencing was performed on an ABI 3700 DNA sequencer (Applied Biosystems). An amended DNA sequence protocol was followed after optimisation due to initial results failing to produce consistent levels of sequence quality from similar PCR bands (see below). Chromatograms were edited and aligned using BIOEDIT version 7.0.5.3 (Hall, 1999).

Amended sequencing protocol

Clean up of PCR products prior to sequencing (EXOSAPIT)

1. Add 2 µl of EXOSAPIT to 5.0 µl of PCR product
2. Transfer to PCR block for incubation using the following conditions
3. 37°C for 15 minutes
4. 80°C for 15 minutes
5. 4°C for 2 minutes

BIG DYE Sequencing Reaction

1. Make up the following reaction mixes for each sample in a 96 well plate:

BIG DYE terminator Master Mix v 1.11 µl	1 µl
Better Buffer	3 µl
Primer (1pMol/µl)	1 µl
Template	0.5 µl
H_20	4.5 µl

2. Transfer to PCR block for incubation using the following conditions:
   i. 96°C for 30 seconds
   ii. 50°C for 15 seconds
   iii. 60°C for 4 minutes
   iv. 4°C for 2 minutes
   } 25 cycles
3. Place at 4°C until you are ready to clean up

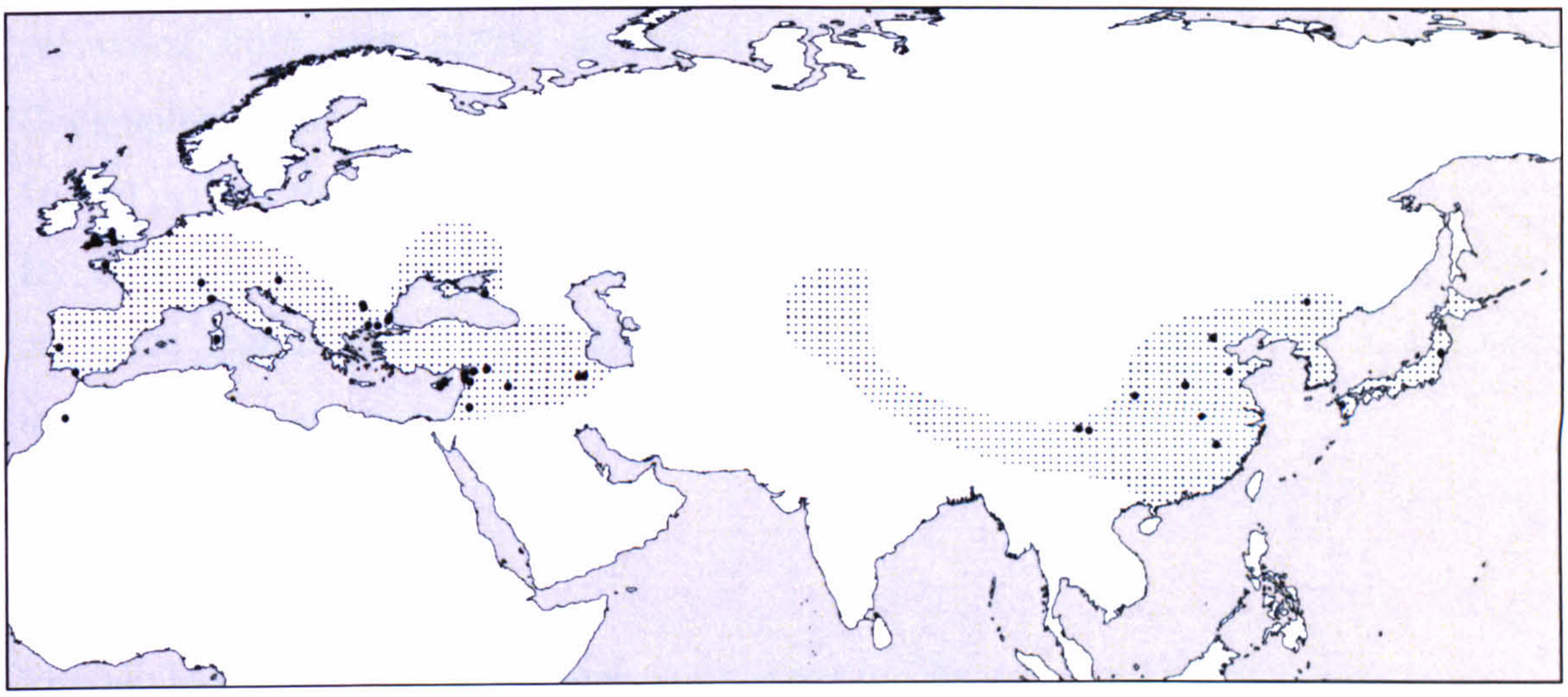
Post-sequencing clean up (removal of unincorporated BIG DYE)

1. Add 60 µl 100% ethanol: 7.5 M ammonium acetate (33:1) to each sample well
2. Seal plate and invert several times to mix
3. Spin (with 09100/09366 rotor) at 3100*g*/4°C for 30 minutes
4. Tip off supernatant immediately onto tissue
5. Spin plate inverted on fresh tissue at 250 g for 1 minute
6. Add 100 µl chilled 70% (v/v) ethanol to each well
7. Spin at 3100*g*/4°C for 2 minutes
8. Tip off supernatant immediately and repeat steps (6) and (7)
9. Tip off supernatant immediately; spin upside down on tissue at 250 g for 1 minute
10. Allow plate to air dry for 10 minutes before re-suspending in 10 µl of water
1. Add 8 µl HiDi formamide to each well of an ABI 96 well plate
2. Transfer samples to ABI plate
3. Denature for 5 minutes at 95°C

Table 13. Sampling locations by country and region, co-ordinates, sample size and collector/supplier of all samples used in this study. Collectors/suppliers of samples are denoted by their initials as follows: AP = Alenka Petrinjak; CD = Christian Dietz, DR = Danilo Russo; DS = Dino Scaravelli; EK = Eugenia Kozhurina; FL = François Leboulanger, GJ = Gareth Jones; GL = Gang Li; JF = Jon Flanders; JJ = Javier Juste; LE = Lazaro Echenique-Diaz, MS = Mozafar Sharifi; MZ = Maja Zagmajster; PB = Petr Benda; RA = Raphaël Arlettaz; SR = Stephen Rossiter; SZ = Shuyi Zhang.

Country	Locality/Region	Easting	Northing	n	Collector/Supplier
Bulgaria (N)	Nanin Kamak	E24:51:22	N43:37:37	5	CD
Bulgaria (N)	Samara Pestera	E25:29:58	N41:24:31	2	CD
Bulgaria (N)	Urushka Maara	E25:01:46	N43:14:44	1	CD
Bulgaria (SE)	Primorsko	E27:45:01	N42:17:15	1	CD
China (NE)	Beijing	E116:19:55	N39:54:25	12	GJ/SZ
China (NE)	Jilin	E126:29:56	N43:46:49	1	GJ/SZ
China (C/W)	Foping (Shaanxi)	E107:59:00	N33:34:00	1	PB
China (E)	Shenxian Cave (Henan)	E113:22:05	N34:41:49	9	GL/SZ
China (E)	Xiaya Cave (Shandong)	E118:07:01	N36:15:00	2	GL/SZ
China (E)	Wanfu Cave (Jiangxi)	E117:01:46	N28:05:47	1	GL/SZ
China (SE)	Youkuang Cave (Anhui)	E115:19:59	N31:10:01	1	GL/SZ
China (SW)	Nanton Village (Sichuan)	E102:13:52	N29:48:33	1	GJ/SR/SZ/GL
China (SW)	Emei Shan (Sichuan)	E103:16:92	N29:34:74	1	GJ/SR/SZ/GL
Cyprus	Cinarli	E33:46:00	N35:19:00	1	PB
Cyprus	Kalavasos	E33:16:00	N34:48:00	1	PB
England (SW)	Buckland	W04:09:57	N51:07:00	4	GJ
England (SW)	Chudleigh	W03:36:19	N50:36:15	3	GJ
England (SW)	Coomb Down	W02:21:21	N51:21:41	5	GJ
England (SW)	Corfe Castle, Dorset	W02:03:03	N50:37:59	2	GJ/JF
England (SW)	Golden Mill	W04:54:50	N50:16:55	5	GJ
England (SW)	Gunnislake	W04:12:33	N50:31:20	3	GJ
England (SW)	Woodchester	W02:16:39	N51:42:39	4	GJ/SR
France (N)	Normandy	W03:00:30	N48:10:56	13	FL
Greece (NE)	Koufovouno	E26:27:13	N41:21:10	2	CD
Iran (NW)	Ghalah Kord Cave (Zanjan)	E48:49:59	N35:45:00	5	MS
Iran (NW)	Kataleh Khor Cave (Zanjan)	E48:15:00	N35:40:01	1	MS
Italy (NW)	Giovo	E08:28:15	N44:25:49	8	DS
Italy (SW)	Campania	E14:41:39	N40:57:13	1	DR
Japan	Sendai, Honshu Island	E140:53:33	N38:15:03	15	LE
Morocco	Talkout, Oued Tessaout Valley	W07:17:00	N31:41:00	1	PB
Portugal	Exact locality unknown	W08:05:05	N39:23:10	3	CD
Russia (W)	Exact locality unknown	E38:02:59	N44:46:01	8	EK
Sardinia	Grotta di Monte Majore	E08:61:67	N40:05:00	1	CD
Slovenia (SE)	Kostanjeviska Jama	E15:43:84	N45:83:86	2	MZ/AP
Spain (SW)	Jerez de la Frontera, Cadiz	W06:08:14	N36:41:12	7	JJ
Syria (N)	Qala'at Najm (Halab)	E38:16:0	N36:33:0	5	PB
Syria (N)	Qala'at Samaan (Halab)	E36:52:0	N36:18:25	3	PB
Syria (N)	Qala'at Salahadin (Lattaqia)	E36:3:0	N35:36:0	1	PB
Syria (N)	Qala'at Sheisar (Hama)	E36:34:0	N35:17:0	3	PB
Syria (N)	Qatura (Halab)	E36:50:0	N36:19:0	1	PB
Syria (E)	Dura Europos (Deir ez-Zur)	E40:43:0	N34:45:0	6	PB
Syria (S)	Bosra (Der'a)	E36:29:0	N32:32:0	2	PB
Switzerland	Vex	E7:23:56	N46:12:42	1	RA
Turkey (NW)	Dupnisa Magarasi	E27:33:22	N41:50:27	1	CD
Turkey (SE)	Çevlik (Hatay)	E35:56:00	N36:08:00	5	PB

Figure 20. Map of sampling localities across the entire range. Shaded areas indicate the current known distribution of *R. ferrumequinum* (adapted from Corbet & Harris, 1991).



Genetic diversity

Genetic diversity was measured for individual sampling areas and for geographic groupings on haplotype diversity (h) and nucleotide diversity (π). Populations were divided up into broad geographical groupings; Europe, west Asia, central/west China, east China, all of China and Japan. As it was only possible to sequence one sample from Morocco this sample was excluded from the analysis. Values for polymorphic sites and the mean number of pairwise differences were also estimated. All calculations were carried out using the software DNASP, version 4.10.6 (Rozas et al., 2003).

Population structure

Analyses of molecular variance (AMOVA) with a non-parametric permutation method of 10,000 permutations (Excoffier et al., 1992, Excoffier, 2003) were carried out in ARLEQUIN version 3.1 (Schneider et al., 2000) to test for the existence of a geographical pattern of population genetic structure. Populations were grouped by the five main five geographical clades produced by the phylogenetic analysis and were assessed according to the degree of differentiation (F-statistic analogues) between regions (Φ_{CT}), between populations within regions (Φ_{SC}) and between all populations (Φ_{ST}).

To test for isolation by distance between populations (Slatkin, 1993), geographical distance was regressed against Slatkin's linearized Φ_{ST} for all samples containing two or more individuals. Linear Euclidean distances (in kilometres) between samples were calculated from their metric easting and northing coordinates using the program Geographic Distance Matrix Generator (version 1.2.1) (Ersts, Internet). A Mantel test of 10,000 permutations was performed using the program IBDWS version 3.14 (Isolation By Distance Web Service http://ibdws.sdsu.edu/ Jensen et al., 2005) to assess the statistical significance in correlation between genetic and the natural logarithm-transformed geographic distance within the samples.

Phylogenetic analysis

The phylogenetic relationship among all uniquely identified haplotype sequences was inferred by implementing a neighbour-joining (NJ) algorithm using PAUP* version 4.0b10 (Swofford, 2002) and using Bayesian Inference (BI) using MrBayes version 3.1 (Huelsenbeck and Ronquist, 2001, Ronquist and Huelsenbeck, 2003). Statistical support for branching patterns was estimated by bootstrap replication (NJ: 1000 replicates). BI was run with four simultaneous chains, each of 1×10^6 generations, sampled every 100 generations. The first 25% of trees were discarded as 'burn-in' to allow for the likelihoods of the trees to reach a steady state for when the consensus is created. The HKY 85+ gamma model of DNA substitution was determined to be the most suitable model for the analysis by applying MODELTEST version 3.7 (Posada and Crandall, 1998) to the dataset (base frequencies : A: 0.3480, C: 0.3201, G: 0.0961 and T: 0.2358; transition/transversion ratio = 30.0713; gamma distribution shape = 0.0174).

Demographic analysis

The demographic history of *R. ferrumequinum* was examined by looking at mismatch distributions (Slatkin and Hudson, 1991) for sequences from the following geographical regions: Europe, west Asia (excluding Russia), Russia, China, east China and Japan. Samples from central/west China and Morocco were not included due to small sample sizes. Populations that have undergone a recent demographic expansion typically show a distribution which is smooth or unimodal whereas populations that have been in a relatively stable equilibrium will show a ragged or multimodal distribution (Rogers and

Harpending, 1992). The observed distributions were compared with the expected distribution under a model of sudden expansion by calculating the sum of squared deviations (SSD) and raggedness index (r). Parametric bootstrapping (10,000 replicates) was carried out in ARLEQUIN to produce the confidence intervals. When a population was found to have undergone a recent expansion, the timing of expansion in generations (t) was estimated from $\tau = 2ut$. Where τ (tau) is calculated as the time to expansion in mutational units and u is the mutation rate per generation for the DNA sequence being studied. Mutation rates of 1.2% and 1.8% per million years (Myr) were chosen as the upper and lower boundaries of the mutation rate for ND2, as previously calculated for the Philippine fruit bat (*Haplonycteris fischeri*) (Roberts, 2006) and New Zealand short-tailed bat (*Mystacina tuberculata*) (Lloyd, 2003). The generation time of *Rhinolophus ferrumequinum* was estimated to be 2 years based on Ransome (1995).

Nested clade phylogeographic analysis (NCPA)

Despite the recent controversy surrounding the use of nested clade analysis (Garrick et al., 2008, Petit, 2008a,b), it was tentatively used as an additional approach to detect genetic/geographical variation and to separate population structure from population history (Templeton et al., 1992, Templeton et al., 1995, Templeton, 1998, Templeton, 2008). NCPA was reconstructed by creating a minimum spanning haplotype network (MSN) based on the 95% statistical parsimony of Templeton *et al.* (1992) using the program TCS version 1.21 (Clement et al., 2000). The MSN haplotype networks were then arranged into hierarchical clades using the nesting algorithm provided by Templeton et al. (1987) and Templeton and Sing (1993) then testing for geographical and genetics differences using 10,000 permutations with the program GEODIS 2.5 (Posada et al., 2000). The NCPA inference key was then applied to nested clades with significant associations using the program Automated Nested Clade Analysis (ANeCA) version 1.1 (Panchal, 2007) to identify clades that have become isolated by distance or by past fragmentation or range expansion (Templeton, 1998) using the latest version of the inference key given by Templeton (2004):
(http://bioag.byu.edu/zoology/crandall_lab/geodis.htm).

Results

Mitochondrial DNA gene diversity and population structure

In total, 31 unique haplotypes were identified from 161 *Rhinolophus ferrumequinum* individuals at 45 different locations based on 1098 bp of the ND2 gene. A total of 109 polymorphic sites (9.9%) were recorded, of which 96 (8.7%) were parsimony informative with a transition/transversion ratio of 30.07.

Haplotype diversity (*h*) was calculated and found to differ both between and within regions (Table 14). In Europe, no variability was seen in 7 of the 11 countries sampled (haplotype diversity = 0). Non-zero values were recorded in Bulgaria, England, France and Spain with two haplotypes recorded in each with the exception of Bulgaria which had three. All haplotypes were separated by a single basepair and, with the exception of England, the main European haplotype was found in each of these countries (Fig. 21). In west Asia, variability was seen in 4 of the 5 countries sampled with 10 haplotypes observed overall. Syria was found to be the most diverse country sampled in this region with 6 haplotypes recorded over 7 locations. The localities sampled in east Asia appear to be similarly diverse as west Asia with variability seen in both China and Japan (Fig. 21). In China, 11 haplotypes were found over 10 localities and, in Japan, 3 haplotypes were observed from the single sampling location. A similar trend is also seen for nucleotide diversity and number of pairwise differences although small sample sizes may give higher values for some of the populations within regions (Table 14).

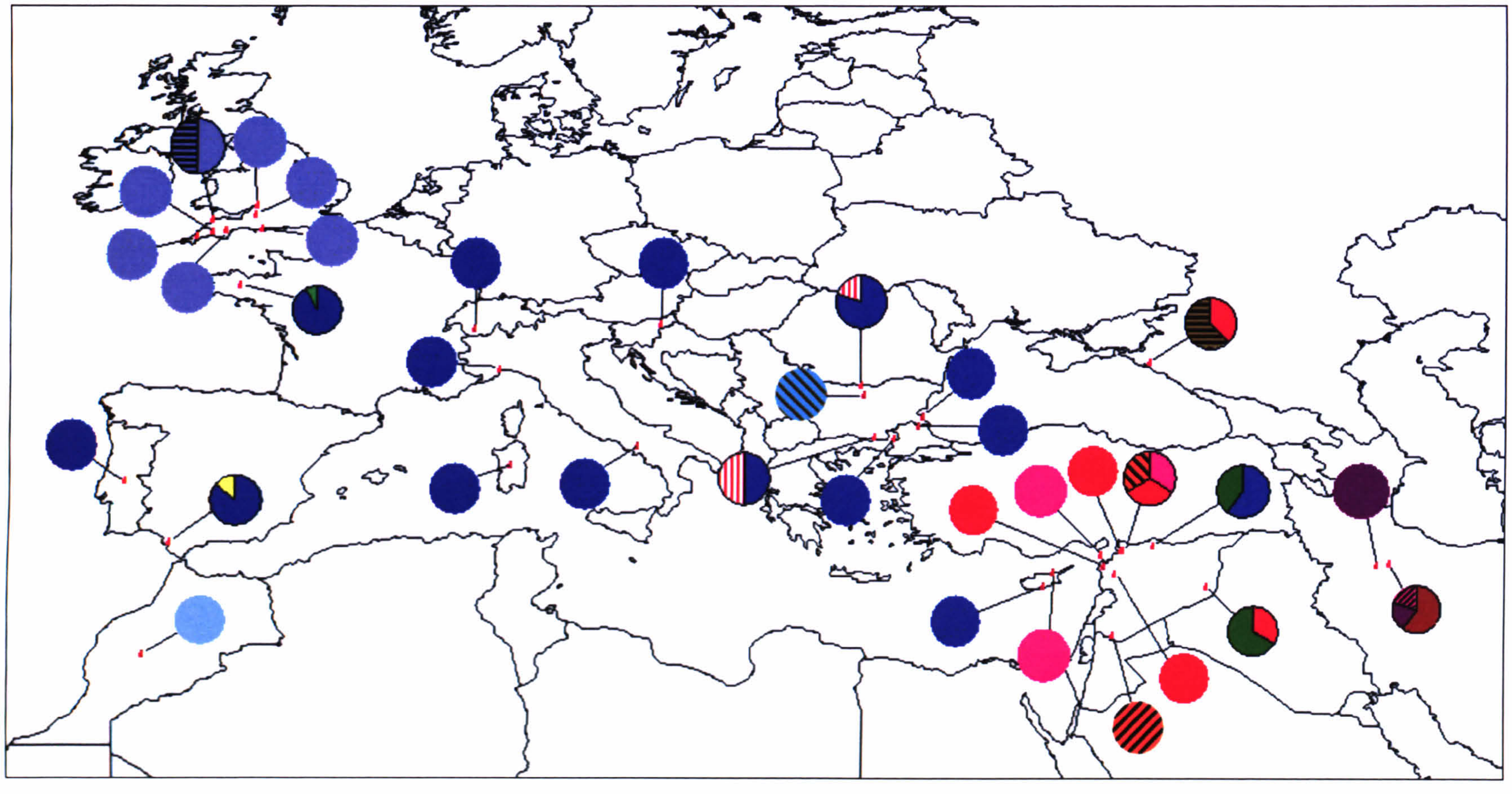
Analysis of molecular variance (AMOVA) revealed significant genetic variance at all three hierarchical levels tested (among regions, among populations/within regions and within populations) ($P < 0.001$), though greatest variation was seen among regions (93.44%) (Table 15). Pairwise genetic distance plotted against geographical distance (Isolation by Distance) indicated significant isolation by distance across the species range (Fig. 22; Mantel test: $b = 23.29 \pm 3.90$, $R^2 = 0.241$, $P < 0.0001$) with the sharp increase in gradient attributable to comparisons between all five major geographical regions (Europe, west Asia, central/west China, east China and Japan).

Table 14. Genetic variability in 26 populations of *R. ferrumequinum* based on 1098 bp of mtDNA. Sample size (*n*), number of haplotypes observed, number of polymorphic sites, mean number of pairwise differences among sequences, haplotype diversity (*h*) and nucleotide diversity (π) are shown. Morocco not shown due to the single sample collected from the region.

No.		Locality	n	Haplotypes observed	Polymorphic sites	Mean no. of pairwise differences	*h*	π
1	**Japan**	Sendai	15	3	2	0.267	0.257	0.00024
2	**China**	Jilin	1	1	0	0	0	0
3	**East China**	Beijing	12	4	6	1.379	0.455	0.00126
4		Shenxian (Henan)	9	3	2	0.611	0.556	0.00056
5		Wanfu (Jiangxi)	1	1	0	0	0	0
6		Xiaya (Shandong)	2	2	20	20	1	0.01821
7		Youkuang (Anhui)	1	1	0	0	0	0
8	**Central & west China**	Emei Shan (Sichuan)	1	1	0	0	0	0
9		Foping (Shaanxi)	1	1	0	0	0	0
10		Nanton Village (Sichuan)	1	1	0	0	0	0
11	**Europe**	Bulgaria	9	3	2	0.611	0.556	0.00056
12		England	26	2	1	0.148	0.148	0.00013
13		France	13	2	1	0.154	0.154	0.00014
14		Greece	2	1	0	0	0	0
15		Italy	9	1	0	0	0	0
16		Portugal	3	1	0	0	0	0
17		Sardinia	1	1	0	0	0	0
18		Slovenia	2	1	0	0	0	0
19		Spain	7	2	1	0.286	0.286	0.00026
20		Switzerland	1	1	0	0	0	0
21		Turkey (NW)	1	1	0	0	0	0
22	**West Asia**	Cyprus	2	2	11	11	1.000	0.01002
23		Iran	6	3	3	1.267	0.733	0.00115
24		Syria	21	6	15	3.638	0.776	0.00331
25		Turkey (SE)	5	1	0	0	0	0
26		Russia	8	2	1	0.536	0.536	0.00049

Figure 21. Haplotype maps from *R. ferrumequinum* from sampling locations in (a) Europe and west Asia and (b) central/west and east China and Japan. Each colour/pattern represents a different haplotype. Pie charts represent the proportion of different haplotypes found in each region.

(a)



(b)

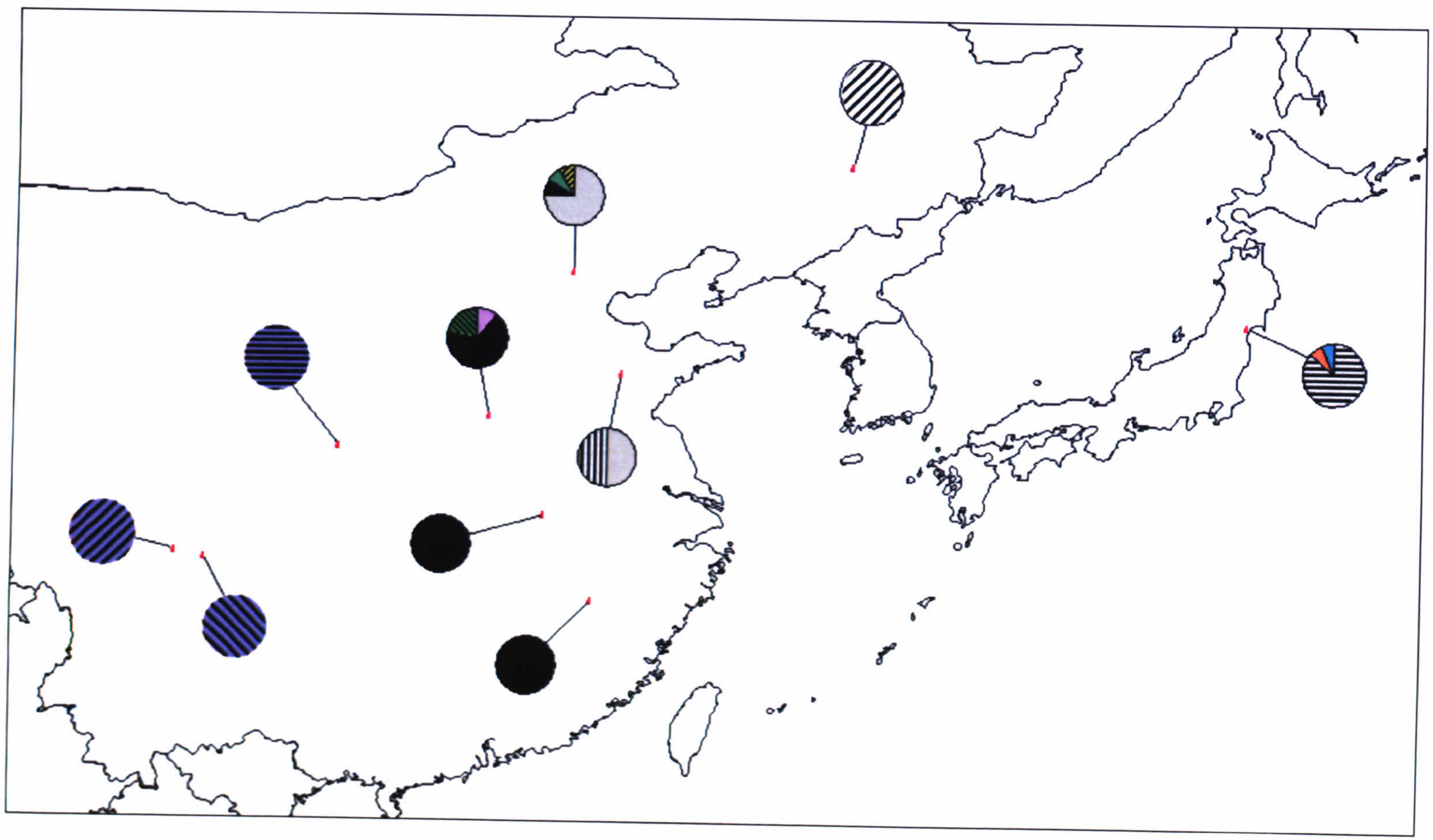
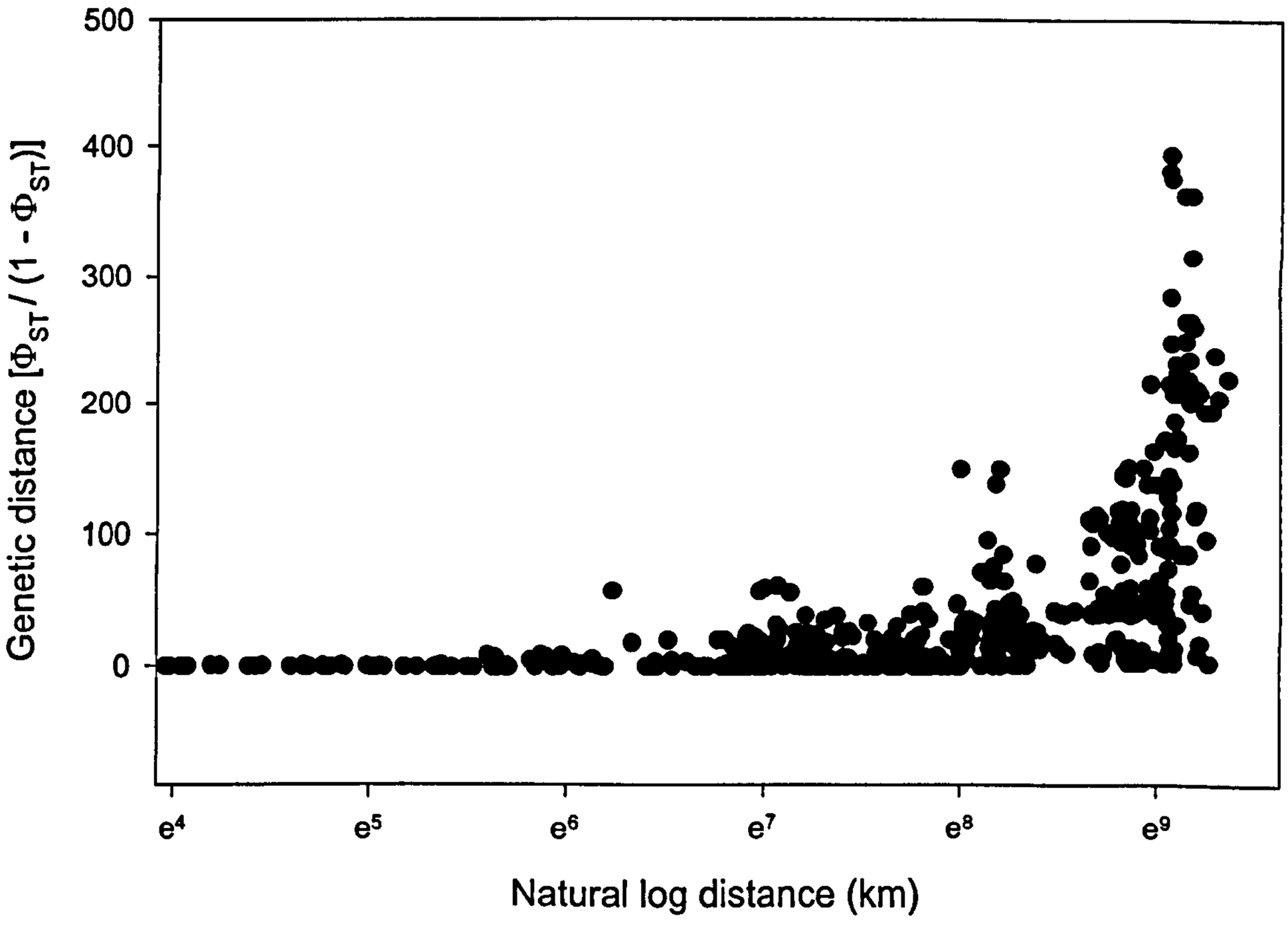


Table 15. Hierarchical analysis of molecular variance (AMOVA) among mtDNA ND2 gene region of *R. ferrumequinum* with different geographical groupings. Regions chosen: Europe, west Asia, central/west China, east China and Japan.

Structure	Source of variation	Variation (%)	Fixation indices	*P* value
Five major regions	Among regions	93.44	Φ_{CT} 0.680	<0.001
	Among populations/within regions	4.46	Φ_{SC} 0.979	<0.001
	Within populations	2.10	Φ_{ST} 0.934	<0.001

Figure 22. Plot of genetic vs. geographical distance for pairwise population comparisons using Slatkin's linearized ΦST to calculate genetic distance on population sizes >2.



Phylogenetic and demographic analysis

Neighbour-joining (Fig. 23) and Bayesian analyses (Fig. 24) produced highly concordant trees each revealing that *R. ferrumequinum* forms a monophyletic lineage with respect to *Rhinolophus cornutus pumilus* (GenBank Accession no. AB061526). In both trees haplotypes grouped into two major clades (Europe/Morocco with west Asia vs. China and Japan). Within these clades there was high bootstrap support (>87%) for five main lineages: Europe/Africa, west Asia, east China, Japan (with 2 samples from east China) and central/west China.

Demographic analyses carried out for each geographic region revealed contrasting population histories. Separate mismatch distributions for European, Japanese and Russian regions failed to reject the model of a population expansion (Fig. 25) ($P_{SSD} > 0.05$) based on the sum of squared deviations (SSD), though only the Russian and Japanese regions were rejected based on the raggedness index ($P_R > 0.05$). In contrast, the multiple peaks produced by the west Asia and Chinese regions were indicative of populations that have remained at a relatively constant size. The time of expansion for these three populations was calculated as c. 40,000 – 60,000 years BP (95% CI 24,000 – 101,000 years BP) for Europe, c. 14,000 - 81,000 yrs BP (0 – 154,000) for Russia and 127,000 – 191,000 yrs BP (22,000 – 265,000) for Japan. Of the three different regions, the population expansion from east China to Japan appears to be the oldest. Estimating the time of expansion of the Russian population from west Asia is difficult due to the large confidence intervals due to the limited number of bats sampled from Russia.

Figure 23. Neighbour-joining tree of the observed haplotypes for *R. ferrumequinum* in this study. Numbers along branches indicate the percentage bootstrap support. Numbers at end of branches refer to the different haplotype groups. The tree is rooted with *R. cornutus pumilus* (# 32).

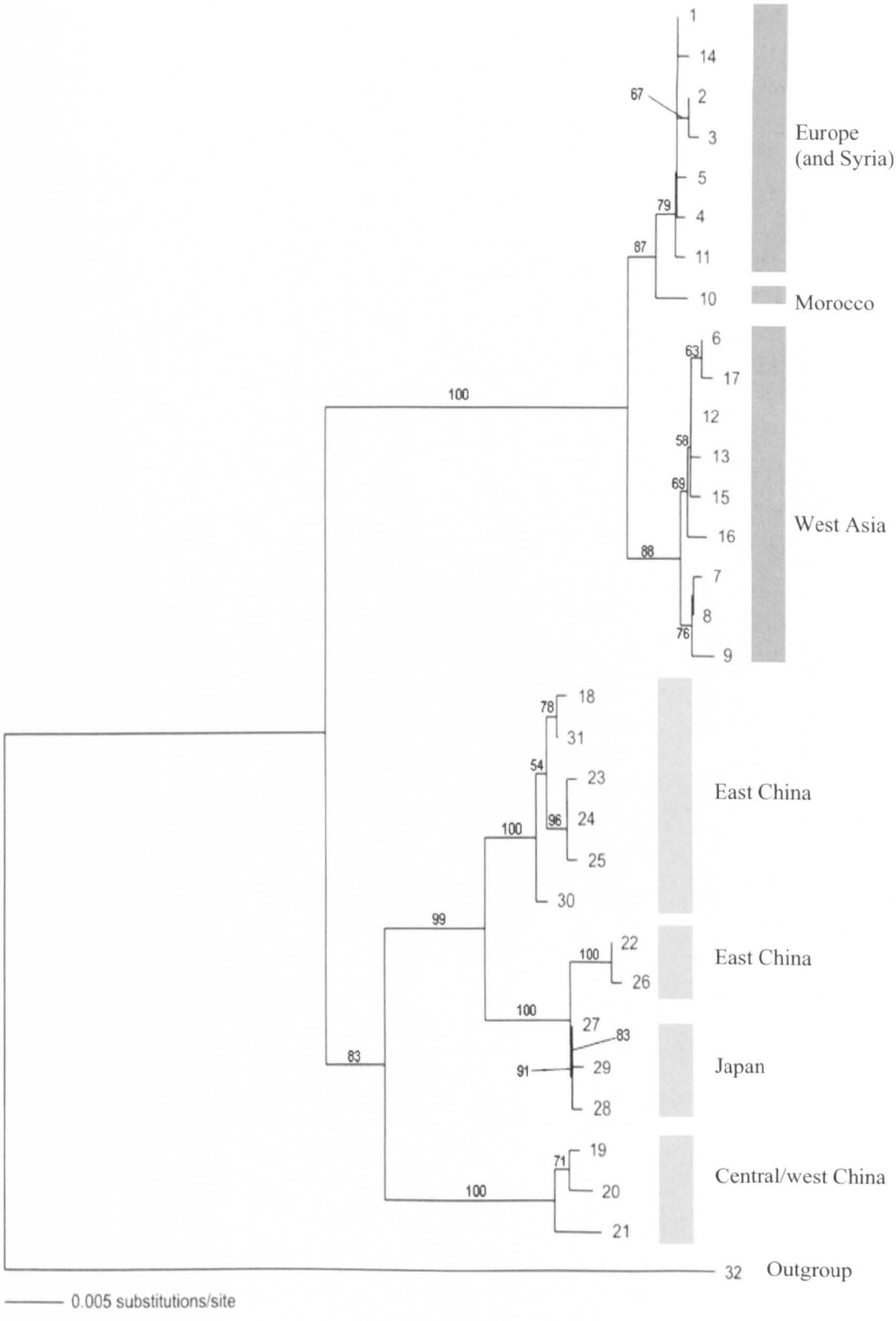
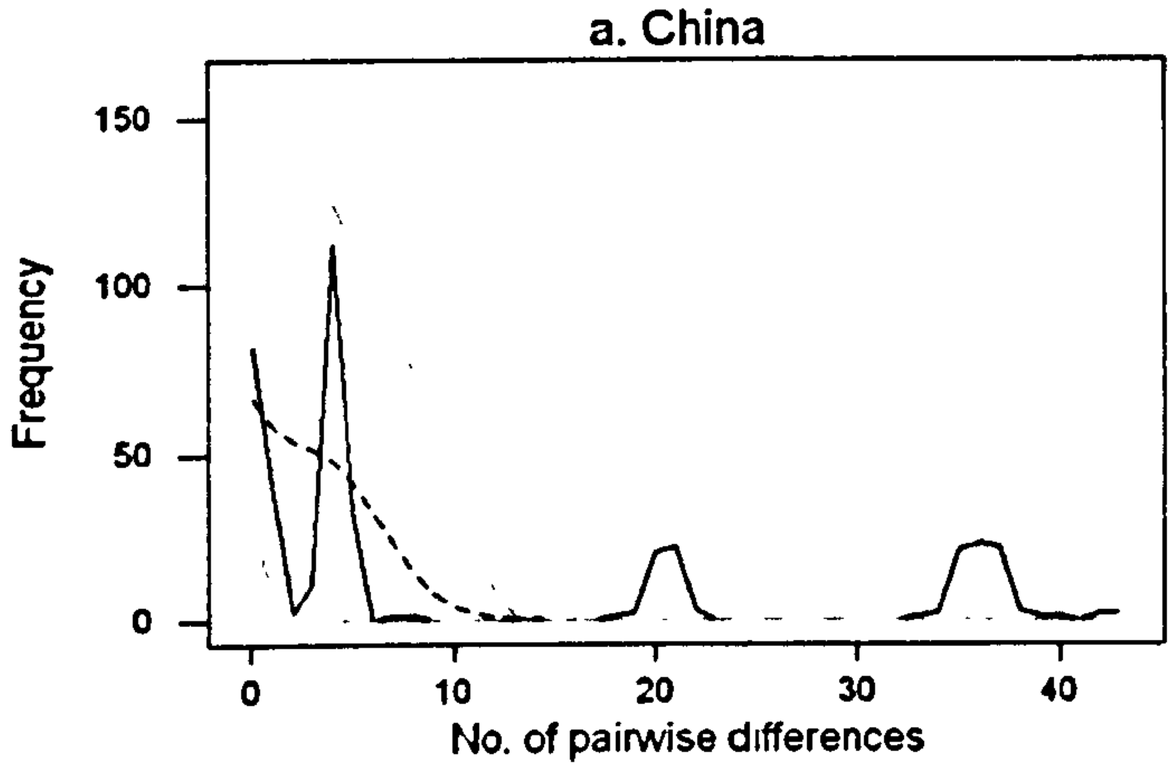
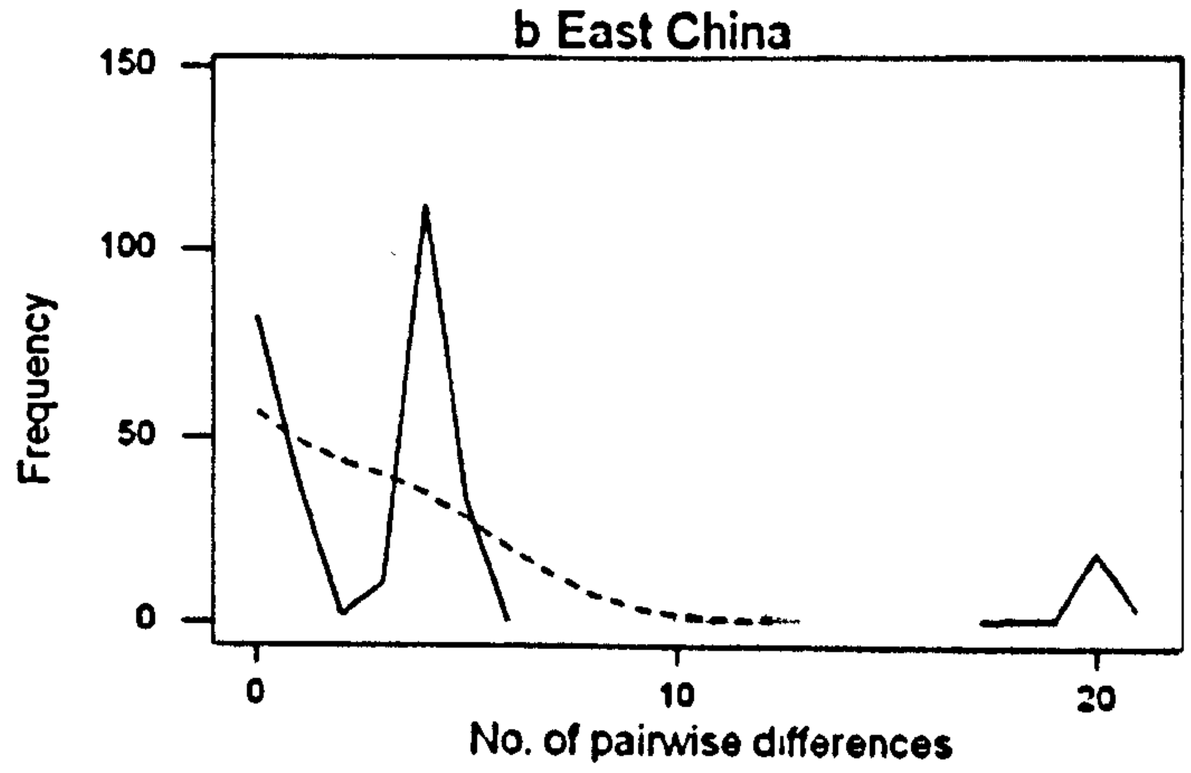
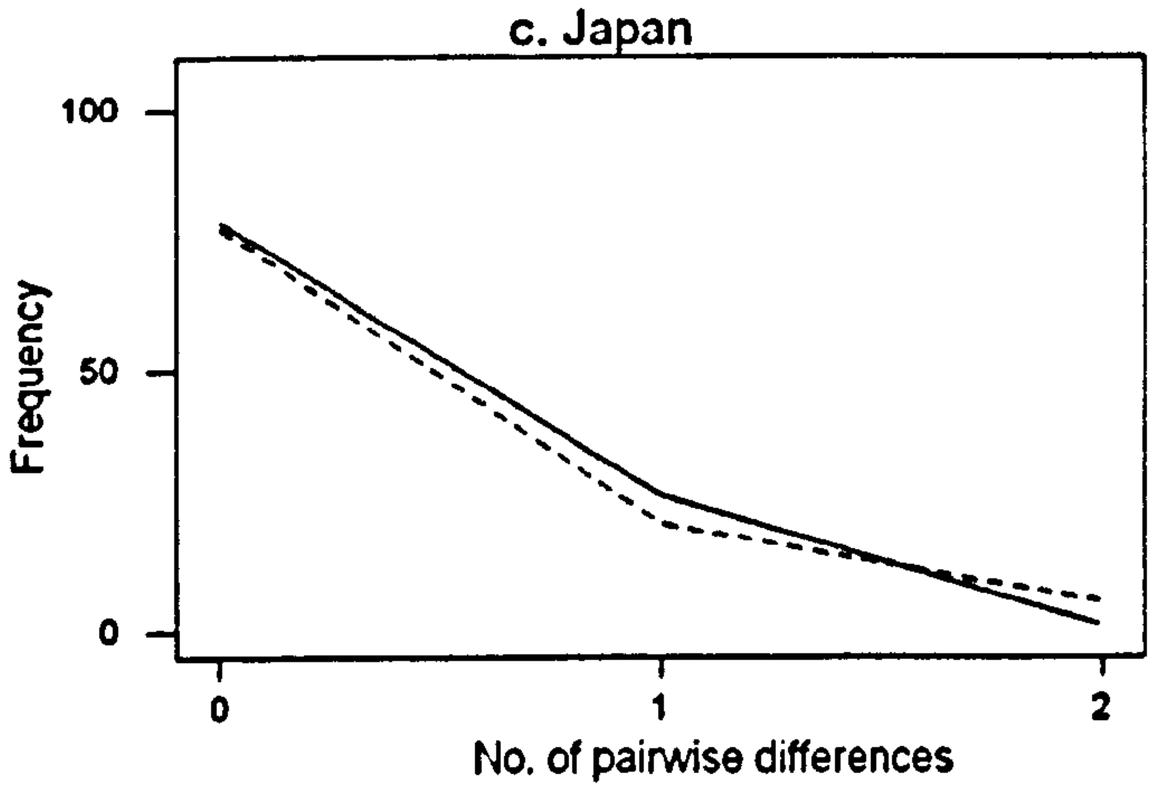


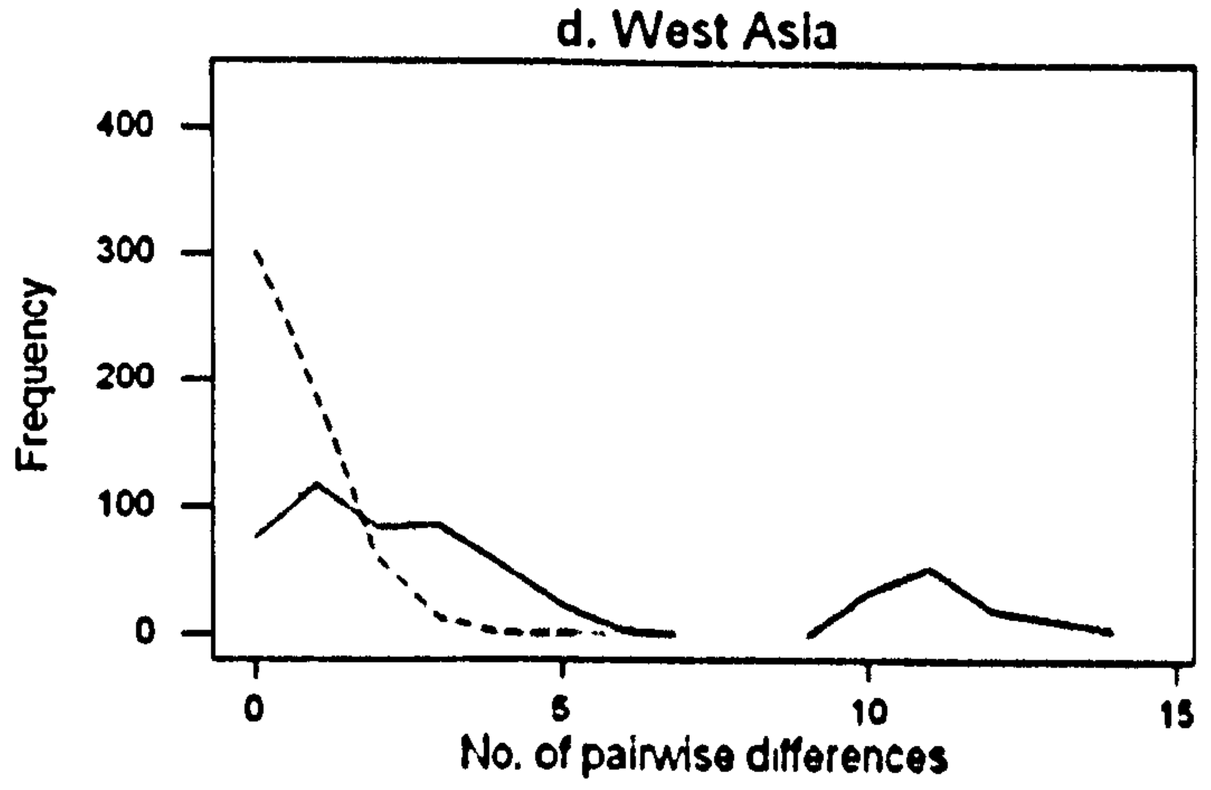
Figure 24. Bayesian distance tree of the observed haplotypes for *R. ferrumequinum* in this study. Numbers along branches indicate the percentage bootstrap support. Numbers at end of branches refer to the different haplotype groups. The tree is rooted with *Rhinolophus cornutus pumilus* (# 32).

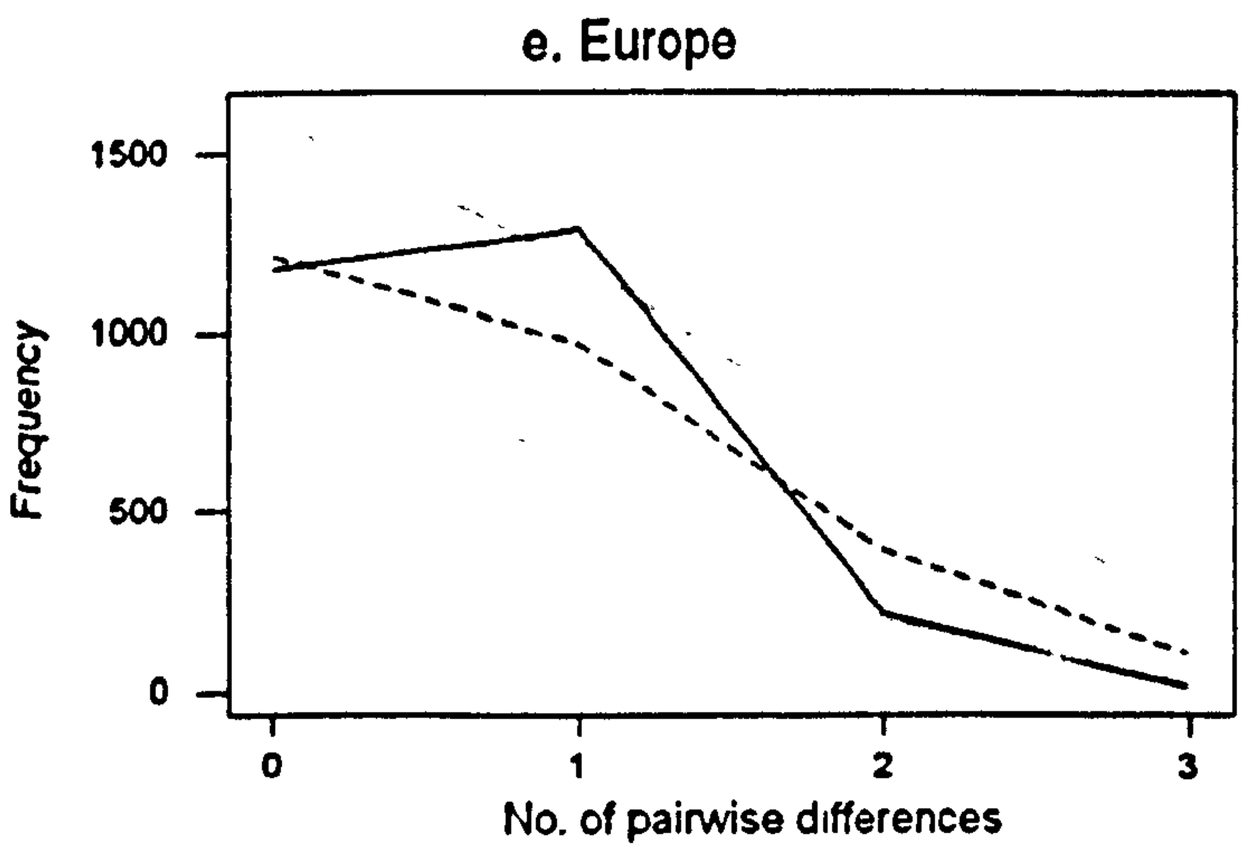
Figure 25. Mismatch distribution of *R. ferrumequinum* for different geographical groupings (a) China, (b) east China, (c) Japan, (d) west Asia and (e) Europe. Solid black lines indicate the observed frequency of pairwise distributions, dashed black lines indicate the expected Poisson distribution under a model of population expansion and the solid grey lines indicate the upper and lower confidence intervals for the expected. Russia is not shown as it has too few pairwise differences.











Nested clade phylogeographic analysis (NCPA)

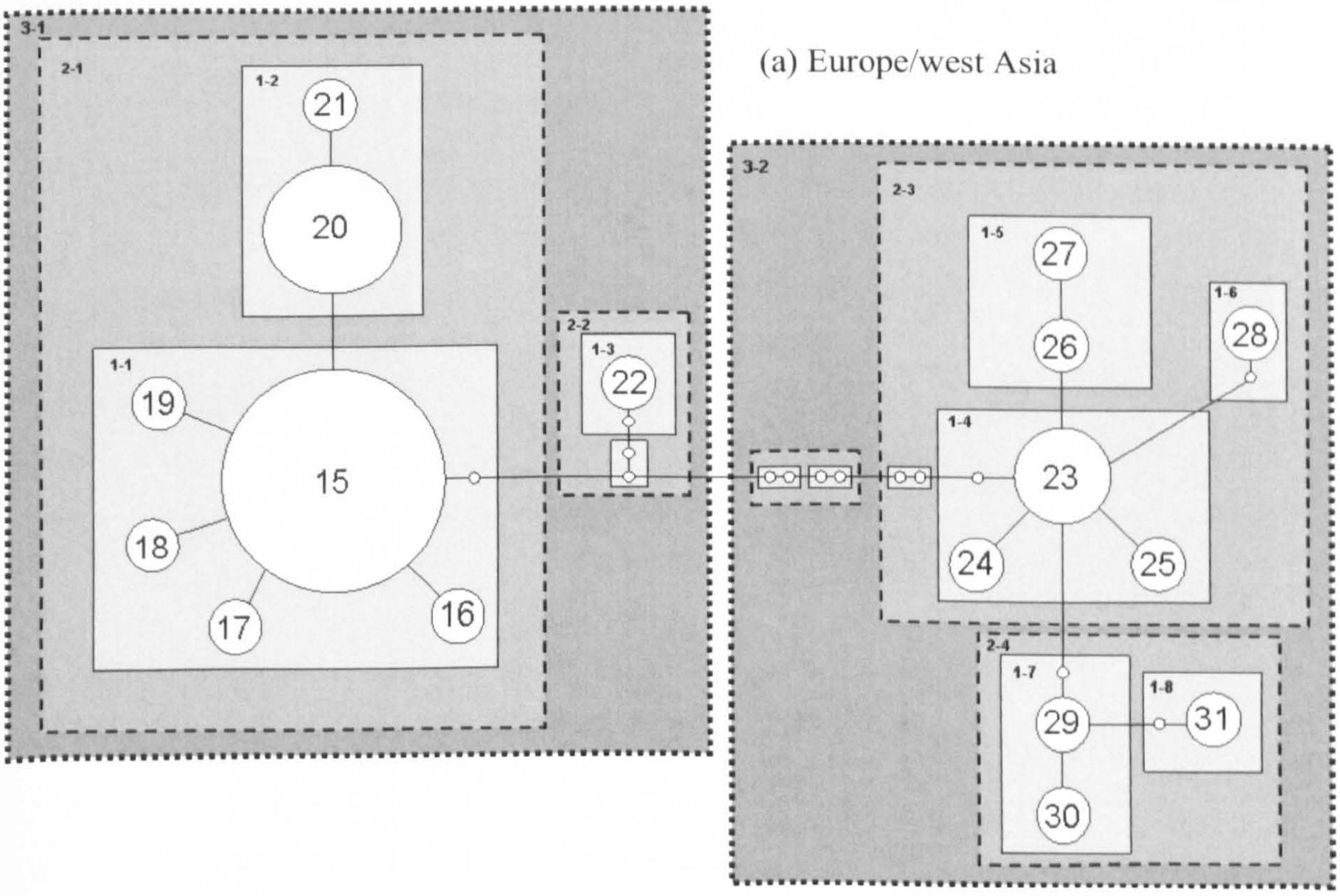
A minimum spanning network, based on 95% statistical parsimony, showed three distinct lineages (Fig. 26) (Europe/west Asia/Morocco, central/west China and east China/Japan). Within these lineages Europe and west Asia was separated at the 3-step 95% haplotype network while in the central/west Chinese lineage the individual sampled at Foping (Shaanxi province) was separated at the 2-step level from the samples collected at Emei Shan (Sichuan province) and Nanton (Sichuan province). The east China/Japan lineage showed the same geographic ambiguity as the phylogenetic trees as two of the bats from sampling sites in east China (Xiaya and Jilin) were more closely associated with the Japanese samples and separated at the 2-step 95% haplotype network from the rest of the eastern Chinese samples.

The nested clade phylogeographic analyses revealed significant geographical associations for European/west Asian/Moroccan clades 1-4 ($\chi^2 = 24.44$, $P = 0.003$), 2-1 ($\chi^2 = 78.00$, $P < 0.001$), 2-3 ($\chi^2 = 60.36$, $P < 0.001$) and 3-2 ($\chi^2 = 38.00$, $P < 0.001$). One significant geographical association was seen in east China/Japan clade 3-1 ($\chi^2 = 17.00$, $P = 0.008$) (Fig. x). The NCPA chain of inference, as constructed by ANeCA (Panchal, 2007) using the key from Templeton (2004) (Table 16), indicates that the geographical associations in the European/west Asian/Moroccan clades 1-2, 2-1, 2-3 and 3-2 have resulted from restricted gene flow with isolation by distance. Interpretation of the geographical association of the European/west Asian/Moroccan clade 1-4 (Syria, Cyprus, SE Turkey and Russia) was of long distance colonisation or past fragmentation. The discrepancy seen in the east China/Japan clade (China/Japan 3-1) was keyed out as the continuous range expansion of bats from Japan to China.

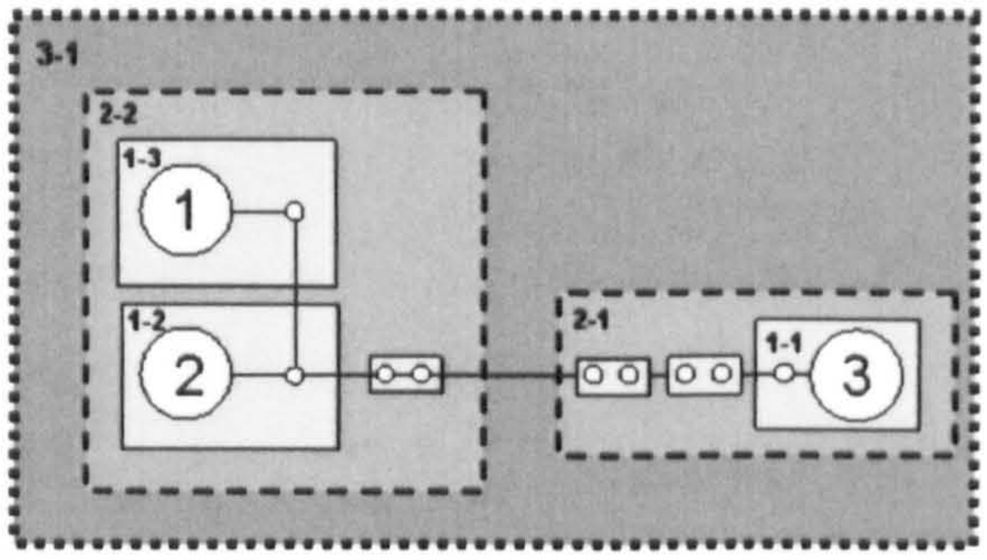
Table 16. Interpretation of the results of the nested clade phylogeographic analysis using the inference key of Templeton (2004). Clades with a significant or near significant association are shown (*).

Clade	Chain of inference	Inferred historic event
Europe/west Asia 1-2	1-2-3-4 NO	Restricted gene flow with isolation by distance
Europe/east Asia 1-4*	1-2-11-12-13-14 NO	Long distance colonization and/or past fragmentation
Europe /west Asia 2-1*	1-19-20-2-3-4 NO	Restricted gene flow with isolation by distance
Europe /west Asia 2-3*	1-2-3-4 NO	Restricted gene flow with isolation by distance
China/Japan 3-1*	1-19-20-2-11-12 NO	Contiguous range expansion
Europe /west Asia 3-1*	1-19 NO	Allopatric fragmentation
Europe /west Asia 3-2*	1-19-20-2-3-4 NO	Restricted gene flow with isolation by distance

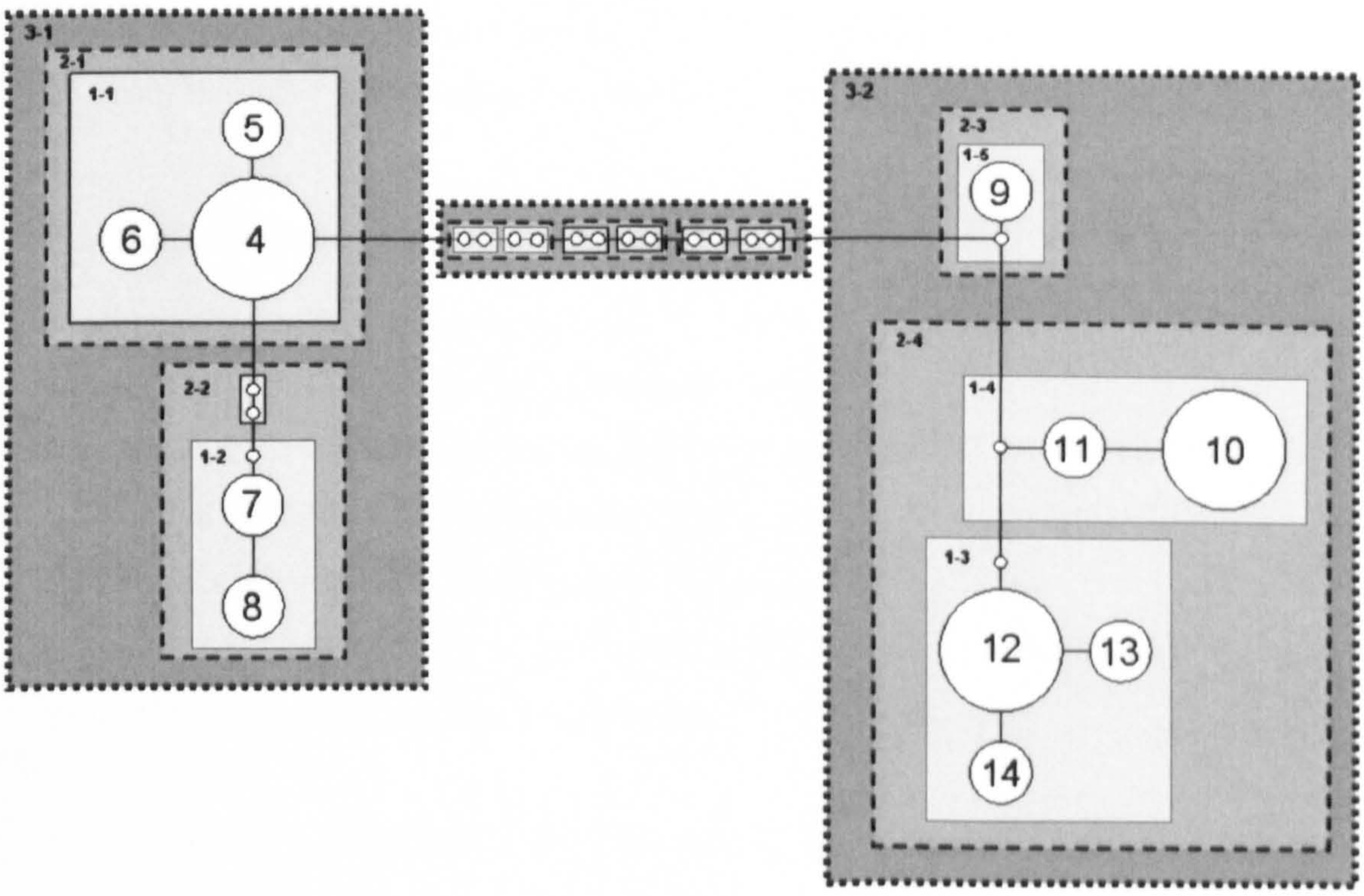
Figure 26. One, two and three step 95% haplotype networks for 161 individuals of *R. ferrumequinum* based on 1096 bp of ND2. Networks are separated when there are more than 12 mutational steps difference between them (a) Europe/west Asia, (b) central/west China and (c) east China/Japan.



(b) Central/west China



(c) East China/Japan



Discussion

Despite the availability of multiple markers to researchers, most phylogeographic studies have typically favoured using mitochondrial genes (Moore, 1995, Avise, 2000), in part because hypervariable markers have been seen to suffer from too much homoplasy to provide sufficiently clear genealogies (Hewitt, 2004a). By using genetic markers with different rates of evolution and sampling across a species entire range these results provide a unique insight into understanding the demographic history of *R. ferrumequinum* that may be shared with similarly distributed taxa. Mitochondrial and nuclear DNA analyses have revealed that integrating a multi gene approach offers valuable information about postglacial glacial expansions and areas of high genetic diversity that may otherwise be lost if only one method is used over a restricted range.

Population history inferred from mtDNA

Phylogenetic and demographic analyses of European and west Asian mtDNA sequences reveals that most of Europe is made up of a single haplotype. Mismatch distribution, haplotypic diversity, tests for isolation by distance and NCPA all indicate that Europe was colonised before the LGM via a single population expansion from west Asian refugia around 40,000 – 60,000 years BP. High nucleotide and allelic diversity and mismatch analysis all point towards there being one, or several refugia in west Asia from which an initial population expansion occurred.

Mismatch analysis on samples from Cyprus, SE Turkey and Russia shows that the role of west Asian refugia seems to be important not only for populations colonising Europe but also for other populations as well. Although the results of the nested clade analysis have to be treated with some caution (Petit, 2008a), they support the mismatch analysis. These results correspond to a number of studies that have postulated that Asia Minor, along with eastern Europe, are areas of ancient glacial refugia and high genetic diversity (Taberlet and Bouvet, 1994, Bilton et al., 1998, Taberlet et al., 1998, Jaarola and Searle, 2002, Durka et al., 2005, Bilgin et al., 2006). A study by Bilgin et al. (2006) found that Asia Minor showed elevated levels of bat diversity with species from Africa, Asia and

Europe all converging on this one area. Their explanation for this was that this area most likely represents the frontier of expansion from this highly diverse region (Bilgin et al., 2006). A similar trend is seen in *R. ferrumequinum* as Syria shows high genetic and allelic diversity compared to other populations and appears to have been the founding population for many population expansions. Sequence divergence data and mismatch analysis of mtDNA data reveal that the Russian population was formed as part of a single population expansion from a west Asian refugium. Due to the limited sample size for this region we are unable to give a reasonable estimate as to when this split from west Asia occurred (c. 14,000 – 81,000 years BP). These dates could put the expansion before or after the LGM and further sampling is needed in this region before any conclusions about whether Russia and Europe were formed as part of the same colonisation event can be made.

Another important region for high nucleotide diversity is China. Similar to west Asia, areas of east Asia must have acted as glacial refugia although due to limited sampling across this region it is difficult to identify how many there were or where they were located. High recorded levels of differentiation in sequence data between central/west China and east China/Japan does suggest that there were at least two refugia in the east. Mismatch analysis and NCPA indicates that the colonisation of Japan by *R. ferrumequinum* was as a result of a single population expansion from east China. High allelic diversity and mismatch analysis both indicate that the colonisation of Japan occurred before that of Europe and Russia (c. 127,000 – 191,000 years BP). This supports the theory that Japan was not connected to mainland China during the LGM (Park et al., 2000b) and acted as a refugium during this time (Adams and Faure, 1997).

The finding that two haplotypes from east China clustered with the Japanese haplotypes raises questions on the taxonomic distinctiveness of these populations. Although the 95% haplotype network and NCPA suggest that the Japanese population has re-colonised two of the locations sampled in east China, this result could also be due to homoplasy or incomplete lineage sorting since their original split or colonisation. The problems associated with homoplasy may not be seen in the European or west Asian samples as the Japanese population's expansion from China pre-dates that of anything seen in the West. If Park et al. (2000b) are correct in that Japan was not connected to mainland China during the LGM, it brings into question how these bats could have got

to these locations if this is due to a re-colonisation process. At present the distance between Japan and east China is ca. 800 km and although sea levels would have decreased during the LGM it is unlikely that migration between the two land masses would have been possible. A more feasible route would have been for the bats to have travelled from Japan to South Korea (current distance between two countries ca. 160 km). This may have occurred by the vagrants being forced over due to a natural event such as a typhoon. The sequence divergence between the two populations (0.47%) compared to the divergence between west Asia and Europe (1.16%) suggests that this event has occurred more recently than the LGM. Further sampling is needed in north east China and, if possible, Korea before this can be resolved. Morphological data supports this explanation of expansion from Japan to east China (via Korea?) as the characteristics of the *R. ferrumequinum* collected from Jilin more closely matched that of Japanese bats (Fukui et al., 2004). The Jilin bat was larger than other Chinese bats and echolocated with most energy at approximately 65 kHz, 10 kHz lower than what is expected for other Chinese bats in the area (G. Jones, unpublished data).

Comparison of mtDNA with microsatellite data

Analysis of mtDNA and microsatellite data produced similar results at a broad geographic scale; i.e. separating populations from Europe, west Asia, central/west China, east China and Japan, but differences did appear when each of these groups were analysed individually, especially within Europe.

By using a combination of mtDNA and microsatellite data this study shows that it is possible to identify two previous colonisation events in Europe, one before and one after the LGM. The first, as supported by the mtDNA sequence data, appears to have occurred during the interglacial period preceding the LGM when the earth would have been experiencing a period of climate warming allowing for the creation of more suitable habitats for many taxa, including *R. ferrumequinum* (for a review of the climatic changes in western Europe see Guiter et al. (2003)). During the last glaciation most of northern Europe would have been covered in ice and tundra forcing *R. ferrumequinum* into one or more refugia in areas such as southwest Europe (Iberia and/or Italy) and southeast Europe (Balkans/Greece) (Hewitt, 1999). Unlike in other European biota such as the bear (*Ursus arctos*), hedgehogs (*Erinaceus europaeus &*

concolor) and grasshopper (*Chorthippus parallelus*) the mtDNA sequence data do not show the accumulation of genetic differences within southern European regions (Hewitt, 1996, 1999). These data thus suggest that either *R. ferrumequinum* survived as one or more refugial populations that remain genetically correlated with each other (either via common ancestry or gene flow). Used on its own, the mtDNA sequence data provides limited information on the resulting colonisation of Europe after the LGM. The use of microsatellite analysis however resolves this as it shows that the re-colonisation of Europe by *R. ferrumequinum* follows a similar trend to the hedgehog paradigm with independent refugia occurring in southeast and southwest Europe (Hewitt, 1999, Rossiter et al., 2007) and not west Asia.

Both markers highlighted the importance of west Asia in not only colonising Europe before the LGM but other surrounding regions as well. Similar patterns were seen with the two types of markers in this region, including showing that Russia formed a distinct group from Syria. Rossiter et al (2007) postulated that this is indicative of a refugium north of the Caucasus mountains but were unable to confirm this as a limited sample size for this region meant that they were unable to reject the possibility that other effects such as population bottlenecks or founder events were influencing their results. Mitochondrial sequence data also point to the same conclusion but due to the small sample size from this region it was not possible to put a confident date on the colonisation event.

There is general concordance in the demographic history between Japan and east China between both types of markers. In this region, mtDNA sequence data proved the most useful because, due to the mutational constraints that operate at microsatellite loci between divergent populations (Goldstein and Pollock, 1997, van Oppen et al., 2000, Queney et al., 2001), a neighbour-joining tree of Japan and China versus Europe based on R_{st} gave erroneous relationships among some samples. Microsatellite data were also unable to be used to support the theory that the Japanese population of *R. ferrumequinum* pre-dated the LGM.

These results have important implications for phylogenetic studies. If this study focussed on mitochondrial analysis alone, it would have inferred a single colonisation event from the west Asia before the LGM. It would not have provided any information

about possible refugia within Europe during the LGM or on postglacial colonisation routes after it. If it had not been for the microsatellite data the low mtDNA variation and NCPA analysis would have rejected the theory of multiple refugia for *R. ferrumequinum* in southern Europe during the LGM. Consequently this study would not have been unable to identify colonisation patterns in Europe after the LGM and compare them with others often identified in this region (i.e. the 'grasshopper', 'hedgehog' and 'bear' paradigms (Hewitt, 1999)). Similarly, although the genetic diversity of the microsatellite data indicated a population expansion from west Asia, its faster rate of evolution limits its ability at inferring events before the LGM. This limitation can be used as an advantage as it provides additional support for the mitochondrial data as it shows there has not been any genetic exchange between Europe and west Asia since the LGM. By using mtDNA sequence divergence data it enables estimates for the time of the west Asia/European split.

As an alternative to using microsatellite markers to identify events since the LGM this study could have used sequenced data from one of the hypervariable (HV) domains in the mitochondrial control region (d-loop). By having a faster rate of mutation than mitochondrial genes HV domains should produce greater haplotypic diversity within Europe. This method, although widely used (Petit et al., 1999, Van Hooft et al., 2002, Ruedi and Castella, 2003, Durka et al., 2005, Bilgin et al., 2006), does have limitations as the presence of R2 repeats (Fumagalli et al., 1996) often reduce the length of the sequence to be compared. An example of this is shown in a study by Ruedi and Castella (2003) investigating at the genetic impact of ice ages on *Myotis myotis*. In this study they were only able to use 307 bp of the HVII control region due to R2 repeats. Even though the HV domains have a higher variability there would be no guarantee that data obtained would have been able to show the level of detail that the microsatellite markers achieved. Out of the 480 bats sampled within Europe, Ruedi and Castella (2003) only found 43 different haplotypes as one main haplotype (H1) was identified in 207 (43%) of the individuals. If this study had sampled bats within Europe so rigorously it may have been able to identify more haplotypes in this region. Instead it was decided to follow similar methodologies to Kotlík and Berrebi (2001) and Culling et al. (2006) in increasing the number of sampling locations at the expense of the sample sizes in order to gain a better understanding of this species' phylogeography. By limiting their study to Europe, Ruedi and Castella (2003) discuss that regions such as Turkey and Syria may

act as a source population for eastern Europe and state that further sampling is needed in these areas. In contrast to the microsatellite data from Rossiter et al (2007), Ruedi and Castella (2003) found that their analysis of 5 microsatellite markers showed little variation over the sampling range due to suspected homogenisation of ncDNA variation over wide areas through male-mediated gene flow. One possible reason for the contrast of results could be due to the number of markers used by Ruedi and Castella (2003) (5) when compared to Rossiter et al (2007) (17).

The importance of phylogenetics in conservation biology

This study is one of the first to compare the concordance between mtDNA and microsatellite data whilst examining the phylogeographic history of a species of bat across its natural range. It shows that the phylogeographic signals obtained from the two markers are different and that only by combining the datasets was it possible to reconstruct the species history through multiple glacial periods. Furthermore, by not limiting sampling efforts to within Europe this study was able to assess the relative importance of European versus Asian refugia. With the ever-increasing number of studies of phylogeography being carried out these findings serve as an important reminder of the limitations of only using one genetic marker or markers with similar rates of mutation and modes of inheritance and only sampling individuals over a limited range.

Biogeography is a powerful tool in understanding the past and current distribution of different taxa and when integrated with conservation biology can help management decisions in the future. Studying a species such as *R. ferrumequinum* provided the opportunity to compare different gene pools and identify patterns of genetic structure and diversity that can help explain what influence different regions such as Europe, the Middle East and Asia have had on shaping this species phylogenetic and demographic history. All these factors are important in conservation biology because by having a distribution that is broadly mirrored by many other European taxa (Corbet, 1978) these results could identify patterns of population history shared by other co-distributed taxa that have previously not been sampled across this range.

General Discussion

In order to provide informed management advice conservation biologists need to have a good understanding of the ecological requirements that are fundamental in explaining how and why a species is found in a given area. This is not a simple question and involves investigating all levels of biological organisation from large scale interactions at a landscape level down to studying the behaviour of individual organisms (Noss, 1990). Advances in technology have facilitated the development of new and quite often specialised techniques that can be used by conservation biologists to help answer some of these questions. Examples of such improvements can be seen in this study with the development of radio-transmitters small enough to be used on bats, acoustic lures to find species that would otherwise go undetected, molecular analyses to look at population phylogeographic history and the use of ecological niche models to predict species distribution over large areas. The major problem with specialisation is that different fields of expertise may not always be used together even though an integrative approach would be more powerful.

The integration of a number of different techniques this study has shown how a range of methods can be used to maximise the information obtained regarding the ecological requirements of different species of bat on the Isle of Purbeck. Also, by carrying out surveys at different times of the year, this study highlights the importance of obtaining detailed information on a species' life history that are sometimes overlooked so that more informed management recommendations can be made.

The work carried out in Chapter 2 presents the first detailed study of *R. ferrumequinum* using non-breeding roosts in Dorset. It is only the third study in the UK to focus on the foraging behaviour of individuals not part of a maternity colony (Jones and Morton, 1992, Duvergé and Jones, 1994) and presents further information on the ecology and behaviour of this species. Consequently, this study was able to reinforce the importance of broad-leaved woodland for foraging bats in the spring. Continual monitoring of Boar Mill over three years showed that non-breeding roosts have an important role in this species' life cycle and may be especially important in a fragmented environment where individuals have to travel large distances from maternity colonies to mating territories

and hibernacula. The inconsistent use of Boar Mill during the summer reflects the need to carry out surveys of buildings throughout the year so that bats are not overlooked. These results show that current management plans need to be updated so that more protection is given to non-breeding roosts and their surrounding areas. At present, there is little information on what should be done to protect *R. ferrumequinum* non-breeding roosts as management plans only focus on conserving maternity roosts (Ransome, 1996, 1997) and hibernacula (Ransome, 2002). If managers use habitat recommendations following the advice more appropriate to maternity roosts less emphasis will be given to broad-leaved woodland and more to pasture. Although pasture land is important, a greater level of protection needs to be given to woodlands in close proximity to non-breeding roosts because, once felled, it will take a long time any woodland habitat to recover. Until new guidelines are produced, the conservation of non-breeding roosts should broadly follow the recommendations already written for protecting the habitat surrounding *R. ferrumequinum* hibernacula (Ransome, 2002).

Identifying the distribution and abundance of a species' is one of the key requirements before any conservation management plans can be made (Primack, 1993). Without this basic information very little can be done to assess the conservation status of a species because none of the ecological requirements needed to identify whether it can persist in a given area are known. The creation of knowledge-based ecological niche models (ENMs) in Chapter 3 proved to be an effective way to help identify areas where *M. bechsteinii* maternity roosts could be found across the south of England. It showed that when there are no true presence or absence data available for empirical models, a subjective method such as expert knowledge provides a more systematic approach than just randomly surveying any given area. A comparison of the knowledge-based model against the presence-only 'MaxEnt' showed that although useful, once the knowledge-based model has lead to the identification of a number of positive locations, more conventional empirical models are preferred. Perhaps more importantly, the findings of this study would not have been possible without the acoustic lure enabling validation surveys to be carried out. The importance of using a variety of techniques to maximise data collection is shown here because without the acoustic lure there would have been no way of knowing the accuracy of the ENMs making them of limited use (Fielding and Bell, 1997). If individual *M. bechsteinii* had not been caught and radio-tracked it would

not have been possible to identify the maternity roost trees which provide further information on the roosting behaviour of this species.

In Chapter 4 the importance of a number of underground sites for swarming bat communities was assessed by catching individuals at the entrance of each site. Although similar studies have been carried out before (e.g. Fenton, 1969, Parsons and Jones, 2003, Parsons et al., 2003a, Parsons et al., 2003b, Rivers et al., 2006), this is the first study to monitor a number of different sites within close proximity of each other on the same night. The results show that the presence of a number of underground sites in a small area does not mean that they should be regarded as a single swarming site (Parsons et al., 2003a, Rivers et al., 2006). A high level of inter- and intra-specific variation between the different sites shows that each site is equally important and the loss of one site could have important conservation implications for a single species. Conservation management plans should aim to protect as many of these sites as possible. If a site needs to be modified to ensure its long term survival then careful restoration works need to be carried out to ensure that its importance for the bat communities using it are not lost. Only by collecting long term data sets of bat activity before and after a site is altered can any assessment be made of the success of the works which can then be transposed into producing effective conservation management in the future.

One of the aims of conservation biologists is to understand why a species is found in a given area (Cox, 1993). Using molecular techniques, Chapter 5 looked at the demographic history of *R. ferrumequinum* to see how past colonisation events and population structure has been influenced by the Last Glacial Maximum (LGM) (Hewitt, 1999, 2004b). Phylogenetic analyses are important because they allow for a better understanding of how different populations are related to one another. This is especially important when studying intra-specific phylogenies of small, fragmented populations such as *R. ferrumequinum*, as barriers to gene flow can be identified and conservation implementations proposed to limit any further loss of genetic variation and inbreeding depression (Rossiter et al., 2000). Samples collected from *R. ferrumequinum* on the Isle of Purbeck belonged to one of only two haplotypes found in the UK. Limited genetic diversity in the UK and the results of nested clade analysis supported the hypothesis (developed from sampling over a wide geographic range) that west Asian refugia were

important during the colonisation of Europe before the LGM. Only by combining the results obtained from mtDNA and microsatellite data was a better resolved demographic history obtained that showed colonisation events of *R. ferrumequinum* before and after the LGM. High genetic diversity in west Asian and China highlights the importance of these regions as ancient populations and emphasises that conservation biology involves protecting genetic diversity within a single species as well as protecting overall species diversity.

By studying new aspects of bat behaviour this work has provided further insights into their ecology that will aid development of more comprehensive management plans in the future. It has also shown that even with relatively well studied species such as *R. ferrumequinum* there are still aspects of its ecology and behaviour that are still unknown. Future research should seek to expand on what has already been achieved for many species as well as carrying out new work on species where relatively little is known. Where expanding on some aspects of a species' biology was not possible in the past, new techniques such as molecular analyses need to be incorporated when they become available.

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